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## EXPERIMENTAL THYROIDISM

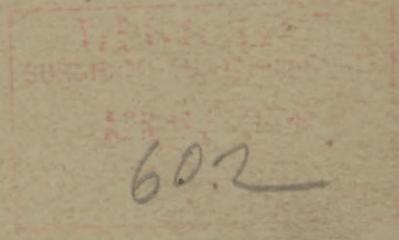
BY

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THE JOURNAL  
OF  
EXPERIMENTAL MEDICINE

EXPERIMENTAL THYROIDISM.\*

BY R. H. CUNNINGHAM, M. D.

(From the Department of Physiology of Columbia University at the College of Physicians and Surgeons, New York.)

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\* This article was received by the editor in August, 1896, but on account of its length and the pressure of other material the publication has been delayed until the present time. In consequence of the active interest and research relating to the thyroid gland during recent years, this delay is much to be regretted. The date of completion of the article should be borne in mind by the reader. (Editor.)

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## I. INTRODUCTION.

With the widespread employment in myxœdema of the thyroid gland and its various preparations that followed the reports of Murray, Horwitz, Mackenzie, and Fox, concerning the beneficial effects of thyroid extract in that disease, numerous other observers have also called attention in the past three years to the fact that in the course of such treatment certain symptoms are liable to arise. The symptoms consist of headache, dizziness, tachycardia, fever, nausea, vomiting, severe diarrhoea, fibrillary tremor and agitation, insomnia, urticaria, incomplete paraplegia, loss of consciousness, and finally general collapse. They are more or less liable to follow either the subcutaneous injection of the extracts, or the ingestion of large doses either of the fluid extracts or of certain commercial desiccated thyroid preparations.

In October, 1893, my attention was personally drawn to this subject by the fact that an adult man (Part I, C, Obs. 1, see below), who had previously taken large doses of unquestionably fresh, raw thyroid gland with absolutely no disagreeable after-effect, promptly developed evident symptoms of thyroid intoxication or "thyroïdismus," after taking daily for three days six five-grain thyroid tablets of Burroughs, Wellcome & Co. The difference in the action of the dried product when contrasted with the inactivity of the fresh gland was so striking

in this instance that I determined to investigate fully the cause of this difference and, if possible, discover the source of the intoxication.

From my first observation referred to above I was led to believe that the toxic symptoms were probably caused by the action of certain toxic substances which had developed in the thyroid glands after their removal from the sheep. This single observation did not sufficiently demonstrate the correctness of the above opinion, and, in order to determine this and a number of points closely related to this subject, I began at that date the extensive series of experiments that are described in this monograph.

In a previous paper \* dealing with one of the many corollaries of this subject I have already referred to the negative results obtained from feeding animals upon large amounts of fresh thyroid glands, but at that date, owing to the incompleteness of some of the comparative intravascular injection experiments and other experiments upon monkeys that are described also in this paper, I refrained from describing the special results that are given here. Previously to that date I had not only expressed the essential points demonstrated by my results to my friend Dr. S. J. Meltzer, but had in conjunction with him performed one experiment that resulted fully in accordance with my previous views upon this subject.

It must be noted that during the progress of this work Prof. Kocher † has intimated that possibly the thyroid intoxication may be due to the decomposition of the thyroid material; and later Lanz,‡ working in Prof. Kocher's clinic, has published his experimental investigations on this subject. This writer experimenting on himself and two friends noticed only an unimportant increase in the pulse rate to follow the ingestion of 20 to 30 grammes of fresh, raw thyroid. Having contrasted the effects of dried thyroid preparations by Merck, Haaf, and Burroughs, Wellcome and Co., upon mice, rabbits, dogs, and men, Lanz concludes that thyroidism consists of two components: "(a) einer Giftwirkung infolge Verarbeitung zersetzen Drüsenmater-

\* *Medical Record*, New York, June 15, 1895.

† Kocher, *Corr. Bl. f. schweizer Aerzte*, 1895, No. 1.

‡ Lanz, *Deutsche med. Wochenschr.*, 1895, No. 37.

ials, (b) einer für die Schilddrüse spezifischen Wirkung. Letzterer, d. h. der Thyreoidismus im eigentlichen Sinne des Wortes, oder besser gesagt die Hyperthyreosis, äussert sich je nach der Provenienz des Drüsenumaterials und je nach der Art des Versuchstieres in verschiedener Intensität ist quantitativ, aber nicht qualitativ verschieden."

I do not consider, however, that the experiments which this observer relates in his article indisputably warrant the latter of these conclusions, for a number of objections may be offered to them. Thus he found that mice die after the ingestion of either the decomposed dried thyroid material or the fresh material, requiring, however, very much larger doses of the latter. In these experiments the so-called fresh material appears to have been in reality a dried preparation that had been prepared as quickly as possible from the recently removed glands by drying *in vacuo*, pulverizing, and administering in the form of what Lanz terms "Totaldrüsenpillen." It is questionable, therefore, whether Lanz really employed the unaltered fresh material, for, before such material can be dried by this means, certain chemical alterations of the various proteid and other substances may readily occur. In fact I shall emphasize later that some of these substances do alter considerably in drying, and that even during a lengthy process of digestion, if large lumps of the unquestionably fresh raw gland have been ingested, a certain quantity of toxic material may possibly form in the central portions of the undigested pieces of thyroid. So exceedingly prone to early decomposition is the thyroid or "neck gland," that even the average slaughter-house butcher is generally acquainted with the fact.

The further consideration of other points dwelt upon by this author is deferred to another section.

On comparing my totally independent results with those of Lanz, I find that some of our experiences accord fully; others, however, do not, and in this paper I have endeavored, where possible, to explain the cause not only of these differences, but also of certain apparent clinical discrepancies that tend more or less still to surround this subject with some degree of uncertainty.

The original plan, therefore, upon which the present paper is

framed is, first, to give a brief historical retrospect of certain previous important observations of others that bear more directly upon the numerous varieties of experiments performed by myself; second, to describe the objects, plan, and mode of research; third, to describe the experiments in definite series; and, finally, after comparing my results with those of others, to state the conclusions. The object being manifold, and the material rather extensive, I have determined for the purpose of description to divide the subject-matter of my experiments into parts, discussing in Part I thyroidism in healthy and diseased animals; in Part II the inquiry is made whether the appearance of the cachexia thyroideomica is hastened or otherwise influenced by the ingestion of altered animal food stuffs; and in Part III are related certain comparative subcutaneous and intravascular injection experiments in which were used various organic substances or mixtures of substances that had been isolated from the thyroid and the thymus bodies.

## II. RETROSPECT OF PREVIOUS OBSERVATIONS.

The first to use the thyroid juice for the purpose of investigating its physiological effect when injected subcutaneously or intravenously into animals seems to have been J. R. Ewald.\* This observer removed the thyroid glands from a healthy dog, minced them, mixed them with 3 ccm. of warm water, and after the expiration of a few minutes pressed out the juice, which was filtered in some instances before using. This juice was then injected into a vein of another dog. Three hours after, this animal fell into a peculiar cataleptic condition which lasted one hour and a half. While in this condition the temperature was not increased or scarcely perceptibly so. In other experiments the subcutaneous method was employed with similar effects in some cases, but in others abscesses and non-absorption of the juice occurred. Ewald also states that similar, though less intense, effects were produced by extracts of the thyroid glands of other varieties of animals.

Previously to the above experiences of Ewald, Pellacani,† at first alone and later in conjunction with Foà,‡ had injected extracts from the liver, the suprarenal capsule, and the kidney of the calf into dogs, rabbits,

\* J. R. Ewald, *Berlin. klin. Wochenschr.*, 1887, March 14.

† Pellacani, *Arch. per le sc. med.*, iii, 1874.

‡ Foà and Pellacani, *ibid.* vii, 1884.

guinea-pigs and frogs. After the subcutaneous injection of the extracts, agitation, vomiting, dyspnoea, somnolence, and general prostration appeared. The next morning the animal was paralyzed and death ensued later. When the extract was injected into a vein, dyspnoea, agitation, rapid heart action at first, which subsequently diminished in frequency, followed. In four hours the temperature had risen, and four hours later it fell, the animal dying shortly after. These writers concluded that these effects were common to many gland extracts. Mattei \* and also Alexander † have disputed these observations. Langendorff,‡ however, repeated Ewald's experiments and found that the subcutaneous injection of thyroid juice produced effects similar to those observed by Ewald. Intravascular injection of the fresh juice produced rapid death. Intravascular clotting of the blood occurred in some of the animals, and in others the coagulability of the blood was lessened. Langendorff, in discussing his results, very appropriately refers to the practically similar effects that Pellacani, Foà, and Wooldridge § had noted as occurring with the extracts of other animal glands.

Alonzo,|| Gley,¶ and Horsley,\*\* repeated Ewald's experiments, but with negative results. Horsley emphasizes the complication of these injection experiments by the presence of the "tissue fibrinogens" of Wooldridge, which are excessively toxic. Halliburton †† and Brodie ‡‡ studied the effects of injections of these nucleo-proteid substances and fully confirm the previous statements as to their toxicity.

In this connection the observations of Wagner, quoted by von Eiselsberg,§§ are interesting. Wagner injected mucin from the salivary glands of the ox into cats and produced agitation, tremors, and tetanoid spasms that more or less resembled those of the cachexia thyroideotomica. The great similarity of these symptoms to those produced by Foà and Pellacani by extracts of liver, suprarenal capsule, etc., is striking and suggests the possibility that a great number of animal substances exist that will produce similar symptoms when injected subcutaneously.

\* Mattei, *Arch. per le sc. med.*, vi, 1883.

† Alexander, *Ziegler's Beiträge*, xi, 1892.

‡ Langendorff, *Berlin. klin. Wochenschr.*, 1889, p. 786.

§ Wooldridge, *Ludwig's Festschrift*, Leipzig, 1887.

|| Alonzo and Ughetti, *Riforma medica*, Oct., 1890, p. 1364.

¶ Gley, *Comptes rend. d. la soc. d. biol.*, 1891, p. 250.

\*\* Horsley, *Virchow's Festschrift*, 1891, and *Brit. Med. Journ.*, Jan. 30 and Feb. 6, 1892.

†† Halliburton, *Journ. of Physiol.*, xviii, 1895.

‡‡ Halliburton and Brodie, *ibid.* xvii, 1894-95.

§§ von Eiselsberg, *Sammlung med. Schriften von d. Wien. klin. Wochenschr.*, No. 4, 1890.

On the other hand, Vassale \* states that, after the intravenous injection of thyroid juice into thyroidectomized dogs, in three animals the symptoms of the cachexia diminished in intensity and the animals were saved for some length of time. Vassale concludes that the juice thus added simply sustains the animal until some accessory gland vicariously assumes the function of the thyroid. Similarly Gley † and Lanz ‡ found that some animals could be thus saved for some time, and the latter of these writers states that thyroidectomized dogs can be kept alive for months with 5 to 10 grm. of thyroid given daily by mouth. When this dose is stopped the tetanic attacks recur; and he also has noticed that the severity of these recurrent attacks diminishes more and more with the lapse of time after the thyroidectomy.

Historically bearing upon the validity of the conclusions that appear to be taught by the experiments of Gley and of Lanz are the well-known observations of Piana, Ewald, Wölfler, Wagner, Carle, Fuhr, Autokratow, Gley and others as to the frequent occurrence of accessory thyroid glands in the aortic and other regions. Gley, and Edmunds § also state that if the parathyroids (Sandström) are left in thyroidectomized dogs the animals live. I have seen young dogs live for several weeks after the removal of both lobes of the thyroid and the parathyroids that were partially embedded in them. In all of these animals either aortic or other accessory glands were found; in some were found several small accessory aortic glands which often were so minute that even one accustomed to the appearance of these bodies might easily overlook them.

In 1894 Ballet and Enriquez,|| endeavoring to produce experimental exophthalmic goitre in two dogs by the subcutaneous injection of the unboiled thyroid extract, claimed that besides fever, tremor and agitation, tachycardia and a swelling of the thyroid gland occurred. This goitre disappeared when the injections were discontinued, and reappeared when they were begun again. The extract used by these observers was a glycerine one, macerated for twenty-four hours and filtered through paper. They state that no abscesses followed the subcutaneous injection of this fluid in more than one hundred injections. The swollen thyroid exhibited ecchymoses on the surface and weighed 3.75 grm. The intestinal haemorrhages they attributed to vasomotor origin from the fact that no

\* Vassale, *Centralbl. f. d. med. Wissenschaft.*, 1891, p. 14.

† Gley, loc. cit.

‡ Lanz, loc. cit.

§ Edmunds, *Proc. of Physiol. Society*, 1895, No. 4.

|| Ballet and Enriquez, *Sem. med.*, xiv, p. 536, 1894, and *Soc. méd. d. hôp. d. Par.*, 1894, pp. 805 and 941.

ulceration of the intestinal mucous membrane could be discovered. The state of the liver and the condition of the blood in the portal vein are not referred to in their paper. From similar experiments on dogs and rabbits Lanz \* attributes these results to septicæmia following the injection of a decomposed preparation, and does not consider them as the symptoms of thyroidism. One of his animals developed an abscess in the parotid gland, and he infers from this that possibly a similar occurrence had misled Ballet and Enriquez. Furthermore the thyroid glands in Lanz's animals are stated to have become decreased in size to a considerable extent. In the hands of Edmunds † no visible effect was produced by the subcutaneous injection of the thyroid extract (mode of preparation not described) into normal monkeys. Of thyroidectomized monkeys, out of eight treated with injections none recovered.

Up to the present I have confined my remarks to the negative and positive results derived from experiments upon the lower animals, but after Murray ‡ in 1891 recommended the subcutaneous injection of a macerated glycerine extract of sheep's thyroids for the treatment of myxœdema, many clinical announcements appeared calling attention to certain dangerous symptoms that were liable to occur during the treatment. Thus Murray § and others agree that the injections were followed in some instances by nausea, flushing, pain in the lumbar region, loss of consciousness, and symptoms of collapse. Mackenzie || and also Fox ¶ gave the extract or the raw gland by the mouth in order to avoid the dangers consequent to the injections. Mackenzie states in his summary that the acceleration of the pulse and the rise of temperature following the administration of thyroid are proportional to the amount ingested. Fox gave both the glycerine extract and the fried mineed gland, and produced in a myxœdematous patient at the expiration of a fortnight profuse perspiration and inability to walk or stand steady.

Kocher and other observers did not observe such toxic symptoms to follow even large doses of the fresh raw gland. Bruns \*\* gave doses of 5 and 10 grm. of fresh thyroid from lambs or calves. In one case only did headache, nausea, and loss of appetite occur. This patient took 46 grm. in 14 days, during which period a loss of 10 kilos in weight occurred.

\* Lanz, loc. cit.

† Edmunds, loc. cit.

‡ Murray, *Brit. Med. Jour.*, 1891, p. 796.

§ Murray, *ibid.*, 1892, p. 449.

|| Mackenzie, *Brit. Med. Jour.*, 1892, p. 940.

¶ Fox, *ibid.*, 1892, p. 941.

\*\* Bruns, *Deutsch. med. Wochensch.*, 1894, p. 785.

In the other cases reported by Bruns no unpleasant disturbances resulted, although the amount of raw thyroid taken was greater. Kocher \* gave 10 grm., and often larger doses, of fresh thyroid without evil effects and unaccompanied by any diminution in the bodily weight; in fact the weight often increased. The only effect of the fresh raw thyroid in doses of 20 to 30 grm. that Lanz noticed in himself and in two friends was an unimportant increase in the pulse rate. The length of time that such doses were continued is not stated nor are the other details of these experiments described in Lanz's paper.

Sonnenburg's † experience with the raw gland in a case of cachexia thyroideotomica in which a small portion of the gland had been left is also very instructive. His patient took 15 grm. of fresh minced calf's thyroid on February 6, 7, 8, 9, 13, 14, 15, 16, 23 and 28, also on March 2 and 8. During the month of March she took fourteen 15-gramme doses of thyroid. The temperature became normal in February and remained so. The weight at the beginning of February was 37 kilos; on the 19th, 37.75 kilos; and on March 3rd the weight had reached 40.2 kilos. Sonnenburg's results differ markedly from the opinions promulgated by numerous later writers regarding the special susceptibility of myxedematous cases to the early occurrence of the symptoms of intoxication during the exhibition of various thyroid preparations.

Béclère ‡ reports the following marked instance of acute thyroidism. 92 grm. of sheep's thyroids having been taken in 11 days by a myxedematous patient, the myxedema symptoms were replaced by those of thyroid intoxication, which consisted of agitation, tachycardia, insomnia, increased temperature and respiration, exophthalmos and incomplete paraplegia. In short a rather severe degree of the so-called thyroidism was produced. No particulars, however, are given either as to the source of the glands or as to their absolute freshness. Nor do the majority of clinical reporters of thyroidism from the raw gland particularize as to the absolute undoubted freshness of the material which had been given. In a number of instances the patient has procured the glands from the family butcher, who often removes them from carcasses that have been hanging in his shop or in his ice-box for one day or more. Such thyroid glands are certainly not fresh in the strict sense of the term. Probably putrefaction has not occurred, but the rapid and well known post-mortem alteration has occurred. In warm weather the dead glands will give an acid reaction even within three hours after death, and

\* Kocher, loc. cit.

† Sonnenburg, *Arch. f. klin. Chir.*, xlvi, 4, p. 857.

‡ Béclère, *Mercredi med.*, 1894, p. 511.

often a perceptible odor is noticeable after six hours in spite of the fact that the glands have been kept in an ice-chest. In fact one of the most vexatious obstacles that was met with in conducting these experiments with the raw gland was the difficulty of procuring large amounts of the perfectly fresh glands and utilizing them within a few moments after their removal.

After the advent of the various commercial desiccated preparations of sheep's thyroids put upon the market by a great number of firms, the occurrence of mild or of severe thyroidism is more frequently noted in the clinical reports. Bramwell, Abraham, Kocher, Leichtenstern, Meltzer, Ewald, Lanz, and a host of English, French, German, and American observers, all relate experiences more or less similar. Briefly summed up, one learns from their reports that the activity, toxic and alleviative, of such commercial desiccated preparations is exceedingly variable, but all of these writers unite in urging the employment of great care at the beginning of the administration of such preparations, in order to avoid, if possible, the effects of overdosing.

Lanz, apparently considering a certain dried preparation as equivalent to the fresh gland, relates several comparative cases as illustrative of his views regarding the causation of at least the greater part of the symptoms of thyroidism by the decomposition products which the various other dried commercial preparations are concluded to contain. For instance, to a case of goitre was given on March 2, two "Totaldrüsengallenpills" (Haaf), each equal to .2 grm., and the dose was increased by one pill each day until March 8. From March 9 to March 13 no thyroid was given, with the result that the pulse fell from 104 to 84. On March 13 ten more pills were given, and repeated thereafter with a daily additional increase of two pills. On March 16 the pulse was 104-108 and temperature  $38.1^{\circ}$  C. No other symptoms occurred. In another case when the progressive dose had reached 10 grm., and this amount had been given twice, the patient complained of headache, lassitude and sleepiness. In other cases as much as 9 grm. of this preparation per day were required to produce the first symptoms of disturbance. With the preparation of Burroughs, Wellecome & Co. even 1 grm. per day could not be borne longer than one week without causing headache, excitement, loss of flesh, etc.

In his experiments on mice Lanz also employed the "Totaldrüsengallenpills" preparation, ultimately with fatal effect, finding that the English preparation in doses of even less than 1 grm. caused death, whereas the mice in some instances required total amounts of 50 grm. of the dried fresh calf thyroid to produce the same effect. These results demon-

stated nothing in my opinion but the fact that the toxicity of the various dried thyroid preparations varies greatly in degree.

Even the latest evidence, therefore, does not satisfactorily answer the question as to whether the absolutely fresh raw thyroid material is toxic or harmless. I submit, therefore, in the following pages the results of my own independent investigations on this subject.

### III. OBJECTS AND PLAN OF RESEARCH.

Bearing in mind the confused evidence that existed in the literature up to October, 1893, as to the toxicity of the thyroid material, and comparing the experiences of previous clinical workers with the results of my first personal observation with the fresh raw gland (page 178), I aimed primarily:

- (1) to determine conclusively whether or no the fresh raw thyroid material is toxic either to man or to the lower animals;
- (2) to investigate the relationship of the symptoms of induced thyroidism to those of Graves' disease;
- (3) to observe whether or no the toxic thyroid or other animal material influences the appearance of the cachexia in thyroidless animals;
- (4) to study the effects of various substances extracted from the thyroid, thymus and other glands upon thyroidless animals, with the possible result of ultimately isolating the active substance that the thyroid body is considered to contain.

Briefly summarized, I proposed to elucidate the above-mentioned objects:

- (a) by producing in various animals acute or chronic thyroidism and noting in every instance the character of the thyroid material employed;
- (b) by also endeavoring to produce in animals a train of symptoms similar to those of thyroidism, employing for the purpose animal material other than the thyroid gland;
- (c) by producing the cachexia thyroidectomica and noting the susceptibility of these animals to the thyroid and other animal material as well as to special substances that had been chemically isolated from this material;

(d) by administering the thyroid material in a certain disease that is claimed by many to be due to over-activity of the thyroid gland.

Lastly, it has been deemed advisable to repeat with some modifications certain intravascular injection experiments of Schäfer \* and Oliver.† My reasons for so doing are given in full in Part III.

Naturally during the progress of my numerous experiments many more or less important and interesting questions in connection with the previous thyroid work of others came to light, but their enumeration is deferred until I have discussed the points upon which they bear directly.

#### IV. OPERATIVE PROCEDURE AND METHODS.

In every instance in which thyroidectomy was performed the animals were anaesthetized with ether and the strictest aseptic and antiseptic precautions observed throughout the operation. In all the intravascular injection experiments ether was also employed. In these latter experiments I frequently administered a large preliminary dose of morphine also, and at the end of each experiment the still anaesthetized animal was painlessly killed by an overdose of the anaesthetic.

In order to produce the intoxications I administered the thyroid or other animal material either by subcutaneous injection or by mouth. My mode of procedure by the latter method was as follows:

*Birds.* Small pieces of thyroid, meat, capsules, tablets, etc., were placed on the back of the tongue and the bird was allowed to swallow them. Liquid extracts, bouillon and powdered thyroid suspended in water were injected into the oesophagus through a rubber tube attached to a syringe of large capacity.

*Rabbits.* Some of these animals would chew the tablets and swallow them. Usually it was necessary to powder them, suspend in water, and give by the tube. The raw material, after it had been minced very fine with a mechanical meat grinder, was mixed with a small quantity of distilled water and injected into the stomach with the tube and syringe.

*Carnivora.* Cats and dogs would readily eat large amounts of the fresh material; the dogs often considerable quantities of even putrid thyroid. Sugar-coated tablets and capsules that had been dipped in syrup were taken with avidity by the dogs; one of the dogs consuming 300

\* Schäfer, Internal Secretion. *Brit. Med. Jour.*, Aug. 10, 1895.

† Schäfer and Oliver, *Jour. of Physiol.*, xvi, 1894; xvii, 1895; xviii, 1895.

tablets in a few minutes and whining for more. Large quantities of powdered material, liquid extracts and defibrinated blood were administered through the stomach tube.

*Monkeys.* In all instances the tube was necessary and only inserted after previously cocainizing the fauces with a momentary spray of 1 or 2 per cent. solution of cocaine. Owing to the ease with which these animals vomit they were kept under close observation for a number of hours after the dose, and if vomiting occurred, the fact was specially noted and the dose not counted.

*Man.* The perfectly fresh raw thyroid material was given always in a very finely minced state, after wrapping in gelatine wafer papers. The dried commercial preparations were taken in the form of capsules or tablets.

In order to produce the intoxications the following varieties of material were used:

- (a) Raw thyroid, fresh, stale, or putrid, from the sheep, pig, calf, ox, and in a few instances from the dog.
- (b) Raw thymus and suprarenal capsules, fresh, stale, or putrid, from the calf and ox.
- (c) Fresh thyroid, rapidly dried *in vacuo*, from the sheep.
- (d) Dried commercial thyroid from the sheep; a great number of different batches from five different firms being tested.
- (e) Meat (muscle, etc.), fresh, stale, or putrid, from the ox.
- (f) Aqueous extracts, glycerine extracts, and bouillon from *a, b, c, d,* and *e.*
- (g) Special substances or mixtures of substances chemically isolated from *a, b, c, d, e* and *f.*
- (h) Defibrinated blood of thyroidless dogs removed during the acme of the cachexia.
- (i) Sundry commercial so-called nuclein preparations and bouillon prepared from them.

Preparation of extracts.—At the outset of the investigation Murray's method was followed;\* later I omitted the carbolic acid and the glycerine, and extracted with water only or with a normal saline solution, in

\* Murray (*Brit. Med. Jour.*, Oct., 1891) prepared the thyroid extract by grinding the sliced glands with bits of ordinary glass tubing in a mortar. After thorough disintegration a fluid drachm of a mixture of equal parts of sterilized glycerine and a five per cent. aqueous solution of carbolic acid was then added, and when well triturated the mixture was macerated for twenty-four hours. At the expiration of this period it was filtered through a double layer of sterilized paper under pressure, and of the nearly clear, pale yellow filtrate 15 minims were employed for each injection.

some instances adding a few crystals of thymol as a mild preservative. These extracts were exceedingly easy to prepare, but rather too crude for aseptic subcutaneous injections. The aseptic fresh extracts were therefore prepared as follows:

A known weight of finely ground material, absolutely fresh—the animal having been slaughtered 10 or 15 minutes previously—was rubbed in a mortar for 15 minutes with an equal weight of boiled distilled water. This mass was placed in a stout, fine-meshed cloth and the juice expressed by a powerful press. This juice was diluted with an equal volume of water to facilitate filtration, quickly filtered through coarse paper by means of a vacuum pump, and finally filtered through a Chamberland candle. By using large quantities of material and employing a number of candles I could procure daily sufficient fresh fluid for my purposes. 2 cem. of this solution was assumed to be approximately equivalent to 1 grm. of the raw material. Throughout the investigation it has been my aim for the sake of simplicity to maintain the strengths of my extracts that were *not filtered* through the Chamberland candles so that 1 cem. of liquid would equal 1 grm. of the raw material. I have thus invariably injected or given twice the noted cubic centimetres of the extracts that had been filtered through the candles. When it is stated that the animal received 15 cem. of the filtered (Chamberland) extract (1 cem. = .5 grm. of gland), I mean in reality 30 cem., or the approximate equivalent of 15 grm. of the raw material.

In preparing the bouillons the thyroid, thymus and meat were also ground as fine as possible, covered with an equal weight of water, and boiled for different periods of time, water being added from time to time to supply the loss from evaporation. After cooling and skimming off the grease the fluid was filtered usually through paper, and the filtrate was then ready for use.

In many instances I found the administration of these bouillons much facilitated by previously evaporating them to one-half or one-fourth of their original volume.

Throughout this article I have applied the term "fresh" to the material only when I was positively sure that such was the case. Our laboratory attendant having witnessed the slaughtering of the animals, the thyroid or other material was immediately removed and forthwith conveyed to the laboratory, where it was prepared for use as rapidly as possible. Generally speaking, within twenty to thirty minutes from the time of their removal I had ground the glands and given them to the test animals.

In certain experiments in which the effects of the fresh material were

compared with those produced by the same material after it had been kept for different periods of time, I proceeded as follows:

Each piece of fresh substance was halved and each half placed in a separate vessel. After weighing, one portion was ground and immediately administered. The remaining unground portion was put in the ice-box for twelve or more hours, then ground, and finally given to an animal as nearly like the preceding one as possible. This method of control consequently enabled me to give the animals approximately the same material in different states of freshness.

Regarding the mode of preparation of the various commercial dried preparations \* that I have employed, naturally I am in the dark. Judging merely from the appearances and odor of the products one would conclude that the different firms employ very different methods. Nearly all of them appear, however, to contain large quantities of common salt, a circumstance that caused considerable trouble in a number of instances with the monkeys.

The description of the preparation of the special substances from the thyroid, thymus and other material is postponed to Part III.

#### V. EXPERIMENTS.

In the following description of my experiments I do not give in detail every experiment performed, for this would make this article unnecessarily voluminous and filled with wearisome repetition. I wish it distinctly understood that the results of the experiments upon which my conclusions are based have been repeatedly obtained, and when I have made an unrepeated observation the fact is distinctly stated. Many of the animals were utilized for a number of different experiments, but a week or more was usually allowed to elapse from the end of one experiment to the beginning of the next, the animal being carefully observed in the interim. Sufficient time was thus allowed for the complete elimination of the preceding preparation.

\* In this connection I take occasion heartily to thank Messrs. Fairchild Bros. & Foster for the large amounts of their thyroid preparations so kindly furnished. Also I am deeply indebted to Mr. Von Holland, of the Eastman's Supply Company of New York, for kindly supplying the greater part of the fresh material that has been used in the course of my experiments.

## PART I. THE PRODUCTION OF THYROIDISM IN NORMAL AND IN DISEASED ANIMALS.

A. *The subcutaneous injection of thyroid and other extracts in normal animals.*

In spite of the previous observations of Foà, Pellacani, Wooldridge, Horsley, Langendorff and others relative to the complications concerned in such experiments, I wished to satisfy myself fully as to whether the absolutely freshly made crude extracts would produce the ultimate lethal effects that these authors ascribe to the various extracts in general. A few tentative experiments were thus performed on rabbits and dogs, but owing to the occasional occurrence of abscesses at the points of injection, I abandoned the crude extracts for the aseptic varieties, viz. those that had been filtered through a Chamberland candle.

Though abscesses very rarely followed the injection of these aseptic, unboiled, aqueous extracts, the ultimate effect of large doses of the various extracts in general was uniformly fatal, some of the rabbits dying in less than one hour from 30 ccm. (= 30 grm. of material), others not until the following day or the day after. Decreasing the doses I found that some of the rabbits died in about six hours from one injection of 10 ccm. of thyroid extract, others withheld this amount for several days and died usually during the night. With doses of 3 to 6 ccm. death did not so frequently occur. Some of the animals withheld these small daily amounts for 6 or 10 days, finally dying during the night, or after manifesting the usual symptoms on the day preceding death.

If the injections were discontinued after the onset of mild toxic symptoms several of the rabbits recovered completely, even though considerable paralytic weakness had developed in the legs.

When the aseptic thymus extract was used my average results were about the same.

With extracts from material macerated from 12 to 96 hours the rabbits succumbed to much smaller doses, the objective symptoms being possibly more pronounced. Similar extracts prepared from the sugar-coated thyroid tablets of Burroughs, Welleome & Co., the only commercial preparation procurable at the time the above experiments were made, appeared remarkably active, 3 grm. often proving fatal to a rabbit in one or two hours.

When very large doses of the fresh aqueous extracts were injected, within 20 minutes in two instances, the animals exhibited fibrillary muscular twitchings, rapid respiration, accelerated pulse, inability to sit up, the animal lying sprawled out on its belly, and marked exophthalmos in many instances. If the dose was small some of these animals recovered; if the dose was large the animal became apparently paraplegic, the paralysis seeming to be due more to muscular weakness than to a central disturbance. Finally, a general paralysis, one or two slight fits, coma, and death supervened. The rectal temperature of some of the animals was investigated and found in the early stages either normal or very slightly increased. If an extract from stale or dried thyroid or thymus had been injected the temperature was usually markedly increased. Frequently in the stage of coma the temperature fell  $1^{\circ}$  F., or even  $1.5^{\circ}$  F. below the normal.

When the dose of the extracts was small and given daily, the animal in the course of a few days became much depressed and refused food. As time elapsed emaciation and great general weakness became prominent. The temperature rose to  $105^{\circ}$  F. or even higher. The hind legs became very weak and the animal appeared paralyzed, the knee jerks often remaining present, however. In two rabbits slight exophthalmos was observed. Finally, a slight, or severe, watery hæmorrhagic diarrhoea developed and the animals promptly died.

The results of my experiments with dogs with all of these filtered, unboiled extracts were practically the same, much larger doses naturally being required to produce a fatal result. Dogs as a rule withstood the injections for longer periods of time, and several of these animals, even after becoming paraplegic, fully recovered when the injections were stopped. One hardy animal withstood 25 ccm. of fresh filtered thyroid extract administered daily, except Sundays, for eighteen days, but finally succumbed with the usual symptoms.

The following experiment will indicate the average result:

*Exp. I.* Four yellow mongrel dogs of about the same weight were chosen. Dog 1 received daily injections of 20 ccm. of fresh thyroid extract and died on the sixth day, having exhibited during life considerable emaciation, great weakness; paralysis of all four extremities, more marked, however, in the hind legs; a temperature of  $106^{\circ}$  F.; pronounced thirst, and numerous bloody evacuations.

Dog 2 received 20 ccm. of a fresh filtered thymus extract daily for eight days and succumbed, manifesting symptoms identical with the above. On the day preceding the death of the animal a slight exophthalmos and considerable conjunctivitis were noticed.

Dog 3 was injected daily with 10 cem. of a filtered thyroid extract prepared from raw material that had been kept twenty-four hours in an ice-box. It died after the fifth injection.

Dog 4 got 10 cem. (= 10 grammes of raw gland) of a filtered extract prepared from some sugar-coated tablets of Burroughs, Wellcome & Co. I note by the way that this particular batch of tablets had produced severe toxic symptoms in man. Result: died twelve hours after receiving the third injection. The symptoms manifested by this dog differed somewhat from those of the other animals. The emaciation was more marked. The fibrillary muscular twitchings were extremely prominent. The hind legs were stiff and kept rigidly extended. Voluntary movement appeared to be completely lost, but cutaneous sensation was present. Considerable cyanosis accompanied by very marked exophthalmos finally set in and after two slight fits the respiration ceased.

The lesions found both in rabbits and in dogs differed only in degree and were as follows:

The composition of the blood appeared to have been greatly altered. Usually very peculiar friable clots were found in the portal vein and its tributaries. Numbers of large or small intestinal ecchymoses were present either in the mucous membrane or beneath the intestinal peritoneum. The liver frequently contained haemorrhages and the surface was often mottled with small haemorrhagic areas. The kidneys were deeply engorged in some animals, but appeared normal in others. Sometimes the medulla of the suprarenal bodies was completely disintegrated and the seat of a haemorrhage. Abscesses of the salivary glands, such as Lanz has noticed, did not occur in any of my animals. The thyroid lobes in three dogs were the seat of superficial or interstitial ecchymoses. These accumulations of blood would naturally increase the weight of these bodies, but a microscopical examination showed that no apparent hypertrophy was present such as Clark, Greenfield, Bamberger, Mackenzie and others state as occurring in exophthalmic goitre in man. In neither rabbits nor dogs did I get the marked diminution in the size of these bodies that Lanz observed in his animals, but the further detailed discussion of this latter circumstance is included in a later sub-section.

From the above I was convinced that subcutaneous injection of the unboiled, crude thyroid extracts is lethal, provided the dose be large, or small and frequently repeated. Having eliminated possible complicating septic and pyæmic effects in the latter instance by using aseptic extracts that had been quickly prepared from absolutely fresh material,

it was concluded that ordinary septicæmia was not a necessary factor in the intoxication.

Comparative experiments with the aseptic extracts of other glands, and commercial nucleo-proteid preparations, have shown me that an intoxication accompanied by the same symptoms and lesions will likewise occur when these latter substances are injected. The symptoms of the so-called thyroidism can thus be produced by the subcutaneous injection of extracts of other glands.

Extracts from material that had been kept for twelve hours or more appeared more active. The extract from the above-mentioned thyroid tablets was similarly exceedingly toxic; but after keeping this and the other extracts until very marked putrefaction had occurred, and refiltering through the candle, the toxicity appeared to become considerably lessened.

Having, therefore, evolved by this means practically no more facts than previous observers have noted, the subcutaneous injection of these complex mixtures was abandoned and I concluded that I could not thus gain definite information either as to the specific toxicity of the unaltered thyroid material, or to the relationship of increased thyroid secretion, or of disturbed thyroid function (Moebius), to Graves' disease.

### *B. Thyroidism from the ingestion of thyroid material.*

#### *1. Administration of fresh glands to normal animals.*

Many clinical observers having established the fact that thyroid juice from the sheep, pig, calf, or young ox, is effective in myxœdema, material from similar sources was employed in the following observations.

Generally speaking, I began the experiments on Mondays and gave daily doses for six consecutive days, skipping the seventh day on account of my inability to procure absolutely fresh glands on that day. During the following week, and often in the third week, the same, or larger amounts even, were continued in order that the test might be as fair as possible. This period of time was deemed sufficient for the effects of possible cumulative action to appear. The rectal tempera-

ture, the pulse, the respiration, the weight, the condition of the thyroid body, and the condition of the eyes, were carefully followed in numbers of animals from day to day. For the purpose of more accurately determining the effect of the thyroid feeding upon the normal thyroid gland, I quickly and carefully exposed and measured the thyroid lobes of several etherized dogs before the administration was begun. On exposing these bodies at the end of the experiment, preparatory to their removal, measurements were again taken *in situ* and the two measurements compared.

Owing to the fact that animal food-stuffs may undergo purefactive alteration in the intestines of rabbits and monkeys as well as in those of man, I invariably introduced the fresh material in an exceedingly finely divided state, hoping thereby to facilitate the digestion and absorption of the entire dose in the shortest possible time.

Obs. 1. A young cock; weight 2520 grm.; pulse 142; temperature  $108\frac{2}{3}$ ° F.; was given 50 grm. per day of fresh sheep's thyroid for sixteen days. The total amount received by this bird was 700 grm. At the end of the experiment the weight was 2530 grm.; otherwise no effect had followed.

Obs. 2. Two rabbits were given 40 grm. of fresh calf's thyroid daily, except Sunday, for twelve doses. Two more rabbits received 800 grm. each of fresh sheep's thyroid in 18 days, in doses of 50 grm. per day. These rabbits decreased from 30 to 75 grm. in weight, but this fact is not surprising, for in order that the stomach should be empty at the time of the administration the animals were allowed to eat only from midday to six o'clock in the afternoon. The effect was negative in other respects. Other similar experiments in rabbits yielded results almost identical with the above.

Obs. 3. Having fed for two or more weeks several dogs with daily doses of as much as 300 grm. of fresh thyroid, I gave to one of these animals nothing in the way of food for six days except a mixture composed of chopped thyroids of the ox, calf and sheep. In every instance the animals gained in weight, one dog increasing as much as 1 kilo. In no animal was the temperature disturbed or diarrhoea produced. The animals were all so lively and jumped about so much that often considerable difficulty was experienced in getting the pulse rate proper to the state of rest. Numbers of careful daily estimations demonstrated conclusively that no acceleration or irregularity had been produced.

Obs. 4. On the normal cat but one feeding experiment was performed. This experiment yielded a negative result after eighteen daily doses of 50 to 60 grm. of mixed sheep and calf thyroids had been given.

*Monkeys.* Obs. 1. Java monkey (*Macacus cynomolgus*) with moderate horizontal nystagmus; weight 1357 grm.; pulse rate 106; rectal temperature  $101\frac{3}{5}$ ° F. 90 grm. of fresh sheep thyroid were given to this animal in three days. At the end of this time the temperature was 101° F., pulse rate 100, and the weight 1354 grm. On the fourth and fifth days the animal received doses of 55 grm. of the fresh raw thyroid, but without disturbance of its general health.

To several other Java monkeys, one Bonnet, one Rhesus, and a Capuchin monkey, doses of 30 to 35 grm. of fresh sheep thyroids were given daily except Sunday for one to three weeks. In none of these latter monkeys did toxic symptoms arise.

Obs. 2. A large Java monkey; weight 2230 grams; temperature  $102\frac{1}{2}$ ° F.; pulse about 160 (animal much frightened); was given 15 grm. of fresh young ox thyroid. The next morning the temperature was 102, the pulse 150; the animal being still much frightened. It was then given 43 grm. more, and daily thereafter the following amounts: third day, 55 grm.; fourth day, 75 grm.; fifth day, 55 grm.; sixth day, 57 grm. (T.  $101\frac{3}{5}$ ° F.; pulse 110; weight 2265 grm.); eighth day, 65 grm. On this date the temperature was  $101\frac{1}{2}$ ° F.; the pulse 110; and the weight after taking the above dose 2280 grm. On the ninth day it received 67 grm.; tenth day, 75 grm.; eleventh day, 57 grm.; twelfth day, 63 grm.; thirteenth day, 67 grm. The animal now weighed 2271 grm.; the temperature was 101° and the pulse 96. The thyroid administration was then suspended and two days later the rectal temperature registered  $102^{\circ}$  F. The large doses ingested by this animal produced therefore not the least manifestation of the so-called thyroid intoxication.

From these experiments it would seem that the temperature of some of the monkeys to whom large doses of the fresh gland were given did appear to diminish somewhat and remain below the normal during the administration. Thus in the monkey quoted in observation 2 the temperature registered  $102\frac{1}{2}$ ° F. before any thyroid was given. During the experiment it seemed to fall progressively, and on the day that the experiment was discontinued had reached 101° F. Two days later it had become  $102^{\circ}$  F., and has apparently remained between this and  $102\frac{1}{2}$  for some weeks.

On the other hand I have noticed that the rectal temperature of many of the monkeys to whom nothing except their usual food had been given would often vary from day to day. The morning temperature of some

of these monkeys would be as low as 99° F., while the afternoon temperature would register from 101 $\frac{3}{5}$ ° F. to 102 $\frac{2}{5}$ ° F. Again, in bringing the animals from their house to the laboratory where the daily doses were administered and the temperatures taken, an unavoidable though short exposure to the open air was necessary. I am unable consequently to determine definitely whether the diminution noted resulted from the thyroid material or was merely an ordinary variation from natural causes.

The practical absence of effect on the bodily weight is noteworthy and contrasts greatly with that produced in monkeys by much smaller though equivalent doses of the dried commercial thyroid.

*Man.* Naturally the experiments described below were conducted in every instance with due precaution, and daily personal observations were made, so that should the least symptoms of the intoxication arise the treatment could be discontinued immediately.

**Obs. 1.** Adult man, aet. 45 years; weight 205 pounds; pulse 80; axillary temperature 98 $\frac{3}{5}$ ° F. Moderate obesity, but otherwise normal. Digestion excellent. Took daily for four days 8 grm. doses of fresh sheep's thyroid, the material being from a lamb that had been slaughtered but a few minutes. No effect was noticeable. From May 22 to June 4 daily amounts varying from 40 to 60 grm. were taken, the fresh thyroid being always finely minced and rolled in one or more gelatin wafer papers. Result: pulse 80; temperature 98 $\frac{3}{5}$  F.; weight 207 lbs.

The fresh thyroid was then replaced with the commercial tablets and the effect of small doses of these is described below.

**Obs. 2.** An adult man, weighing 190 lbs., took daily except Sunday eighteen 16-grm. doses of fresh calf's thyroid. During and at the end of this treatment no perceptible alteration either of the pulse rate or the temperature could be determined. The man gained one pound in weight, however.

The question may arise as to whether these men really received the thyroid gland and not some other gland, such as thymus or salivary glands. Starr.\* Owen † and others relate such occurrences, and in fact such a mistake is rather liable to occur if the choice of the material be left entirely to the discretion of the family butcher. The thymus, "neck sweetbread," or "throat bread," is well known to the average butcher, but the thyroid or "throat glands" are not so generally known, and are in fact often regarded by certain butchers as bits of muscle. I am fully satisfied that all the men for whom I have prescribed the fresh thyroid

\* Starr, M. A., *Med. News*, Dec. 15, 1894.

† Owen, David, *Brit. Med. Journ.*, Feb. 16, 1895.

did get the correct thing, for the slaughter-house butchers who supplied the above men had removed numbers of thyroid glands for my personal use and were well acquainted with the two lobes. Moreover, at my request, the above men brought me from time to time samples of what they were taking.

From the above observations it appears that normal cocks, rabbits, cats, dogs, monkeys and men manifest no toxic symptoms whatsoever after the ingestion of large amounts of the finely ground absolutely fresh raw thyroid substance. Further, no exophthalmos, tremor, increased temperature or acceleration of the pulse could be detected, and the body weight was diminished, and that immaterially, in rabbits only.

## 2. *Thyroidism from stale or dried material.*

*Rabbits.* (a) *Administration of thyroid.* Obs. 1. Several sheep's thyroids were halved and one of the portions was put in the ice-box for twenty-four hours. Of the fresh portion 50 grm. were immediately given at 10.30 A. M. to a rabbit. Of the second or stale portion 50 grm. were also given to a second rabbit. The first rabbit manifested no ill effects, but the second died during the night, evidently having had a severe diarrhoea. The experiment was repeated, but 30 grm. only of the stale material were given to the second rabbit. In this instance the first rabbit showed no visible disturbance; the second, however, died in about twenty-four hours, exhibiting diarrhoea, marked weakness of all four extremities, dyspnoea and cyanosis, exophthalmos and coma, followed by death.

Obs. 2. A large rabbit was given 30 cc. of a water and glycerine extract from glands twenty-four hours old. The death of the animal followed in six hours, after it had manifested the usual symptoms except exophthalmos. A smaller rabbit that had received 50 grm. of the same material in the fresh state on the previous day showed nothing.

A third rabbit was given 20 ccm. of a glycerine extract prepared from glands that had been kept a similar length of time, with the result that on the following morning the animal was dull, took but little food, had an increase of 1.5° F. in temperature, and had lost 20 grm. in weight. After another 20 ccm. dose of the same extract it succumbed during the night.

Obs. 3. Exp. 1. Rabbit; weight 1680 grm.: given 1 grm. (11 grains = 1 sheep's thyroid?) of a dried commercial preparation. Two days

later the animal weighed 1540 grm.; it was dull and refused food, and the temperature had risen  $1^{\circ}$  F. 3 grm. of the same preparation were administered in the afternoon and the animal died during the night, leaving signs of having had a very severe haemorrhagic diarrhoea. The weight of the body after death was 1380 grm.

Exp. 2. Brown rabbit; weight 1705 grm.; temperature  $101\frac{2}{3}^{\circ}$  F.; was given 6 grm. (6 grains = 1 average thyroid) of a desiccated commercial preparation (No. 220005) from another firm. The next day the animal was dull and did not take its food; temperature  $102\frac{2}{3}^{\circ}$  F. Its weight was now 1680 grm. The dose was not repeated and the animal recovered in a few days.

Exp. 3. Rabbit; weight 1700 grm.; temperature  $102^{\circ}$  F.; was given 16 grm. ( $2\frac{1}{2}$  grains = 5 grains of sheep's thyroid) of one of the earlier preparations of desiccated thyroid from a third firm. As no effect followed in twenty-four hours, the dose was repeated with an equally negative result. It should be noted that I had given 5 grain doses three times a day of this particular sample to man for the treatment of obesity, but without producing the desired effect; nor did diarrhoea, headache, pyrexia, etc., occur during a two weeks administration of this preparation.

Exp. 4. Large white rabbit was given 10 sugar-coated tablets of another unnumbered preparation (each tablet = 5 grains fresh gland) from the preceding firm. These tablets were finely pulverized and given as usual through the stomach tube. Result, death in twelve hours.

Exp. 5. Rabbit given three sugar-coated tablets obtained from a fourth firm. Result, death in four hours, preceded by paralysis of the hind extremities, cyanosis, some exophthalmos, rapid respiration, pulse about 280 per minute, two very slight general convulsions, just after the second of which the respiration ceased.

To rabbits, therefore, comparatively small amounts of either aqueous, stale thyroid extracts, or of commercial desiccated thyroid usually prove very toxic. I write "usually," for the toxicity of the different trade preparations varies very greatly. Thus the equivalent of 1 grammie of raw thyroid of a preparation by one firm will in many instances cause death in a rabbit, whereas many times that amount of another firm's preparation may produce slight symptoms only, which may completely disappear in one or two days. Different lots even from the same firm vary very much; some of the lots appearing to be but very slightly toxic, while others are excessively so.

(b) *Administration of thymus.* Wishing to see if identically the same results could not be produced in these very susceptible animals by means of other glands, meats, etc., I made a number of observations to determine this question. The following observation will indicate my results in general from these comparative administrations.

Obs. 4. A number of absolutely fresh lamb's thymus bodies—in many instances that of the calf was used also—were halved and separated into two portions, one of which was put aside for a known number of hours; the other was minced or made into bouillon, and fed to a rabbit, but in every instance with a negative result. After forty-eight hours or more the former portion was similarly prepared and fed to other rabbits. In some instances both the raw material or the concentrated bouillon from it produced death in from two to six hours, the animals exhibiting symptoms usually identical with those produced by very much smaller amounts of the stale thyroid. Usually, however, I found it necessary to keep the thymus glands on ice for a few days longer for marked toxicity to develop. I do not mean to give the impression that these thymus glands had become putrefied, in fact such was not the case, and were one to judge by their appearance and odor they would be considered still fresh enough for human consumption. Indeed, in my experiments with rabbits it has seemed to me that when a great amount of putrefaction has occurred in both the thymus and thyroid material, the bouillon, extracts, etc., appear to be somewhat less toxic. No doubt putrefactive processes alter the chemical structure of the chief toxic substance or substances.

The effects of administering the desiccated commercial preparations of thymus gland were not studied, but on one occasion I administered a dose of thirty-two grm. of a commercial nucleo-proteid \* preparation prepared from thymus and other glands, to a strong healthy rabbit, without consequent ill effect.

(c) *Administration of meat extracts.* Closely related to the results obtained in this series of experiments on rabbits to which stale preparations of thymus and of thyroid were given, are the observations of Kemmerich,† Bogoslowsky ‡ and others. The former of these writers called attention to the fact that in addition to the salts, certain organic substances

\* This nucleo-proteid preparation was prepared from various glands, of which the thyroid was one. Chemically, the preparation contained a considerable quantity of iodine.

† Kemmerich, *Pflüger's Arch.*, ii (1869), 49.

‡ Bogoslowsky, *Centralbl. f. med. Wissensch.*, 1871, 497.

are present in meat extracts and are the cause of the cardio-excitant action that results from the ingestion of the meat extracts. Bogoslawsky, repeating Kemmerich's previous experiments, similarly found increase in the pulse rate and also a rise of temperature. Both fully agree that such extracts are excessively toxic to rabbits, usually producing death within a few hours. Kemmerich gave an extract equivalent to 1333 grm. of horse-flesh to a rabbit which rapidly began to exhibit the following symptoms: dullness; respiration increased from 120 to 180; pulse rose from 240 to 400 and became stronger; a stage of depression then supervened and the animal soon lost the power of maintaining the upright position, the hind legs appearing paralyzed; later violent dyspnea and convulsions terminated the rabbit's existence. The pathological changes consisted of gastro-enteritis, hyperæmia, and many small gastro-intestinal ecchymoses. In other words, Kemmerich's rabbits all died with a chain of symptoms that are practically similar to those that may be produced in rabbits, and also in monkeys, by administering the stale or the dried thyroid material. The pathological changes found in all of my poisoned rabbits fully accord with those described by Kemmerich, but to satisfy myself fully of the correctness of the observations of these writers I repeated their experiments, using, however, ox-meat instead of horse-meat.

Briefly stated, a quantity of absolutely fresh meat was procured from a neighboring slaughter-house and the extraction of four or five pounds of this finely minced meat begun within a few minutes after the death of the ox. As in the case of the raw thyroid material, half of the original quantity was put aside on ice for a number of days, then extracted by boiling and the bouillon given to rabbits. To sum up, the results in this series of experiments were as follows:

In no instance, after repeated trials, did I succeed in producing any toxic symptoms in rabbits by means of the bouillon from the *perfectly fresh* meat, even if the amount of the concentrated bouillon was equivalent to as much as five pounds of meat. After keeping the meat on ice for 10 days or preferably for 14 days, it being still sweet, the bouillon from it proved usually very toxic, causing death generally in from two to four hours, usually preceded by exophthalmos with or without nystagmus. Dyspnea and slight convulsions also frequently occurred.

Similar extracts from good soup meat cut from carcasses that had hung fourteen days or more proved in some instances toxic, and in others not. In one experiment the extract was made from one pound of apparently good soup meat purchased at a third-rate butcher-shop and given to a rabbit, with the result that the animal died in less than an hour.

The symptoms exhibited by these animals poisoned by the stale-meat bouillons and the lesions found after death were similar to those presented by the animals poisoned with the thyroid preparations; but, for the meat to develop its full toxicity to rabbits, it was necessary to keep it very much longer than the thyroid or the thymus, not long enough, however, for a noticeable odor of putrefaction to develop.

The herbivorous horse and cow, according to Brinton,\* may be brought to eat fish and flesh, but had the above-mentioned toxic substance developed in the flesh in considerable quantities, no doubt these animals would present the same degree of susceptibility to the meat extracts that is known to exist in the sheep.

*Birds.* Having fed a cock with large quantities of fresh thyroid gland, later with extract of fresh meat, and finally with extracts of stale meat, with absolutely negative results as regards the bodily weight and temperature, I administered to this bird large quantities of preparations of commercial thyroid, one gramme of which had produced fatal results in monkeys.

*Obs. 1.* Cock; weight 2520 grm.; temperature  $108\frac{2}{3}$ ° F; received daily the following preparations: 1st day, six 5-grain thyroid tablets † of Burroughs, Wellcome & Co.; 2nd day, six similar tablets; 3rd day, twelve ditto—weight 2500 grm.; temperature  $108\frac{2}{3}$ ° F. Feathers fall out readily; 4th day, 10 grm. of Parke, Davis & Co's desiccated thyroid; 5th day, 20 grm. ditto; 6th day, 30 grm. ditto; 8th day, 20 B. W. & Co's tablets (another lot); 9th day, 50 ditto; 10th day, 36 grm. of P. D. & Co's thyroid; 11th day, 32 grm. of Fairchild's thyroid; 12th day, 180 grm. of raw sheep's thyroids, three days old; 13th day, 60 grm. of putrid ox thyroid. Weight 2540 grm., temperature  $108\frac{2}{3}$ ° F.

The effect was thus negative. I have noted that the feathers of this bird fell out in great numbers, but as it was kept in a room maintained at a high temperature owing to the presence of monkeys, probably no special significance should be attached to this occurrence.

*Cats.* Owing to the difficulty met with in administering the dried preparations to these animals, but one experiment was performed,

\* Brinton, *Cyclopaedia of Anat. and Physiol.*, v, 1859, p. 388.

† Chemical examination of small quantities of all the above commercial preparations, some remnants of which I still possess, reveals the presence of iodine, and presumably in the form of thyro-iodine of Baumann.

namely, I gave to a cat 100 B. W. & Co's thyroid tablets at one dose, but without disturbing the general health of the animal in the least.

*Dogs.* In none of the numerous experiments upon normal dogs or upon previously splenectomized dogs could I succeed in producing the slightest sign of the intoxication. Even in the cases in which these animals were fed for periods of a week or more on nothing but sheep's thyroids, no alteration of the temperature occurred. Some of the animals, although thin and in very poor condition at the beginning of this diet, gained flesh rapidly and at the end of the experiment were in far better general condition than at the commencement.

Before any thyroid was administered to two of these dogs I exposed the thyroid lobes in each and carefully and quickly measured them. After the animals had been fed upon large quantities of fresh, stale and dried thyroid, and finally for a week upon nothing but stale thyroid, the dogs were anaesthetized, the lobes exposed and measured *in situ*. The latter measurements practically coincided with those at the beginning of the experiment, and thus no evident alteration in the size of these normal glands had followed the ingestion of the large quantities (300 to 600 grm.) of mixed thyroid.

This non-diminution in the size of the thyroids in these two dogs does not correspond to the results that Lanz \* got by injecting rabbits and dogs with the thyroid juice. This observer states that owing to the extraordinarily inconstant relative size of the thyroid in rabbits he exposed and measured them before beginning the injections, and also during the period of their administration. Considering that possibly this repeated exposure of the glands may have given rise to errors, owing to the fact that the normal thyroid might, like the goitrous thyroid, diminish in size on exposure to the atmosphere, Lanz devised the following procedure to meet this objection: Two rabbits of the same litter were chosen and one was injected, but without previously exposing and measuring the thyroids of either animal. After a certain number of injections had been made both the rabbits were killed and their thyroids compared. The thyroid of the injected rabbit proved to be one-third smaller than that of the uninjected control animal.

It appears to me, however, that from this inconstant relative size of the thyroid, judging from my personal experience in both rabbits and dogs, the latter method is certainly open to greater objections than the

\* Loc. cit.

former, provided the one initial aseptic operation is rapidly performed, care being taken to disturb the blood-vessels and lymphatics of the bodies as little as possible. When a number of exposures are made one can readily imagine that damage, either directly to the gland or to its blood supply, would be far more likely to occur, and result in some degree of atrophy of the gland.

*Monkeys.* Obs. 1. Bonnet monkey; weight 1260 grm.; temperature  $101\frac{3}{4}$ ; pulse 110. Given 8 grm. of sheep's thyroid that had been kept in the ice-box for twenty-four hours. It should be noted that on the previous day 35 grm. of this same lot had been given to another monkey without effect.

Result: The next morning, considerable depression; refused food, moderate diarrhoea, thirst; rapid and irregular heart's action, about 200 per minute; temperature  $104^{\circ}$  F. The dose was not repeated and the animal recovered in a few days, during which considerable emaciation occurred.

Obs. 2. Exp. 1. To a Java monkey, weighing 1358 grm., temperature  $101\frac{1}{2}$  F., pulse about 96, that had been fed two weeks before on fresh gland with a negative result, was given 3 grm. of an unnumbered desiccated thyroid preparation from a certain firm. As this produced no effect by the next morning the animal was given 10 grm. more, and finally 12 grm. of the same preparation on the third morning. Having exhausted my supply of this particular preparation the experiment was discontinued and the animal observed for ten days. No ill effects resulted.

Exp. 2. To the same monkey six 5-grain tablets (each tablet = 5 grains of raw thyroid) of another firm were given. The next morning the temperature was  $103^{\circ}$  F., the pulse 124, and the animal not so lively as usual. It was then given six more tablets. On the following day the temperature was  $103\frac{3}{4}$  F., the pulse 180; great thirst, considerable agitation, slight diarrhoea, emaciation, some exophthalmos, and marked nystagmus. No more tablets were given. 4th day, animal very ill, severe diarrhoea, great weakness, crouches all the time and seems unable to support itself in the upright position. Temperature  $99^{\circ}$  F. and pulse 140. 6th day, bloody diarrhoeic stools, great weakness, and the hind legs apparently paralyzed. Temperature  $98^{\circ}$  F.; pulse about 260; respiration 68. The animal lived throughout the day and night in a partially comatose condition and died early on the following morning.

Autopsy. Marked gastro-enteritis accompanied by numerous small and large submucous and subperitoneal ecchymoses. Mesenteric glands swollen and much congested. Acute nephritis. Liver very friable and

seat of many ecchymoses. Blood in portal vein clotted, while that in the inferior vena cava and cavities of the heart was still fluid. The thyroid gland was not enlarged and appeared unaltered microscopically.

Exp. 3. Java monkey; very lively and somewhat frightened. Weight 1995 grm., temperature  $102\frac{1}{2}$ ° F., pulse 180. Given 1 grammie of a thyroid powder from a third firm. On the next morning the animal was dejected, scarcely noticed its food, but was excessively thirsty, and had a very moderate diarrhoea. Temperature  $105\frac{2}{3}$ ° F.; pulse about 210. The dose was not repeated. On the third day this monkey was slightly better and took its food with some persuasion. The temperature was  $105^{\circ}$  F., and the pulse 160. The diarrhoea had disappeared. On the morning of the fourth day it was found lying on its side markedly agitated and squealing. Voluntary motion in the hind legs seemed to be lost and moderate exophthalmos and nystagmus were present. Shortly after several slight fits occurred and death ensued.

Autopsy (four hours after death). Weight of body 1725 grammes. Stomach, small intestines and mesentery the seat of a number of ecchymoses. Many minute haemorrhages in the liver. Blood in the portal vein clotted; that in the heart and inferior vena cava still fluid. Thyroid body normal.

Exp. 4. A previous monkey, to which 3 grm. of this same preparation were given, exhibited within two hours considerable fibrillary muscular twitchings; slight exophthalmos; paralysis of the hind legs, apparently spastic in character, the knee jerks as well as sensation to slight pin pricks persisting; excessively irregular and rapid cardiac action; rapid and shallow respiration; rectal temperature  $102\frac{2}{3}$ ° F. ( $101\frac{2}{3}$ ° F. normal for this animal). Several slight fits occurred, in one of which there was slight momentary opisthotonus, with considerable rigidity of the extended hind legs. Throughout, the animal seemed to be perfectly conscious, and when small pieces of bread were placed in its mouth tried to chew and swallow them but was unable to do so. In thirty minutes collapse, coma and death occurred.

Autopsy (five minutes after death). The usual haemorrhagic areas in the intestines and liver, none in the stomach. The cerebral pia mater was deeply congested and also that of the cord. A peculiar tube-like thrombus about half an inch in length was present in the portal vein, but the blood in the other large veins and heart appeared abnormally fluid and of a laky tint.

Experiments were made in which a number of different lots from numerous firms gave practically similar results. I was unable to determine which firm's preparations were most toxic, for the toxicity of dif-

ferent lots from the same firm varied much, one lot proving excessively toxic, while another would produce toxic symptoms only after fairly large daily doses had been given for a period of several days.

All these toxic dried preparations produced pretty much the same pathological changes, but the symptoms induced by some of them in several monkeys differed in a few instances, one of which has been described in experiment 4.

*Man.* My experiences as to the effects of glycerine extracts and dried commercial thyroid upon man are essentially similar to those of other observers. Very severe intoxication has not been observed by me, probably because the effect of the thyroid was always carefully followed and the preparations stopped or decreased as soon as the earlier toxic symptoms appeared.

In many instances I gave portions of the preparations that had produced these toxic symptoms to monkeys, rabbits, and other animals, with the result that the monkeys and rabbits invariably exhibited severe signs of this intoxication.

Loss in weight, pyrexia and acceleration of the pulse occurred in man only when the dried preparations were given, never with the finely minced fresh raw thyroid material.

In man, as in the lower animals, it was also found that the toxic activity of all these dried preparations varied considerably. The susceptibility of the healthy individual seems to vary also, but not very markedly.

**Obs. 1.** An obese individual took two 5-grain B. W. & Co's tablets three times a day for three days with only an immaterial increase in the pulse rate. After four days had elapsed another lot from the same firm was given, and one of these tablets taken three times a day produced in four days severe headache, dizziness, a temperature of 102° F., pulse 96, insomnia for one night, and a moderate diarrhoea. Having completely recovered from the above by the end of a week, 4 grains (11 grains = 1 whole sheep's thyroid) of Parke, Davis & Co's dried thyroid were given three times a day for a period of three weeks. From this preparation the pulse increased to 88 in the first week, but fell to 80 in the second. One or two slight diarrhoeic attacks occurred at the end of the first week, but none in the second. The temperature was not apparently affected. In

the third week, on increasing the amount to 6 grains three times a day, the pulse rose to 90, the temperature to 101° F., and the occasional attacks of diarrhoea returned. 5-grain doses were then given and continued for three weeks longer. At the end of this time 16 pounds had been lost.

Other preparations of thyroid from this firm have not, however, been always so well borne, even one-grain doses three times a day giving rise to disagreeable symptoms in some people.

From Armour's thyroid in doses of three grains three times a day I have seen distinct toxic symptoms arise by the following morning in a fleshy female who had taken larger doses of a preparation by Fairchild without the occurrence of any ill effects. My average experience with this preparation is indicated by the following observation.

Obs. 2. A man weighing 191 pounds, who had previously taken eighteen 16-gramme doses of fresh raw thyroid without effect, was given 3 grains three times a day of Armour's thyroid. On the sixth day the pulse had risen from 78 to 94 and the temperature to 99° F. The above quantity was continued and at the end of the second week the pulse was 100, the temperature 101° F. Nausea, moderate headache and slight diarrhoea were complained of. The dose was reduced to 1 grain per day and the above symptoms disappeared. In three weeks the weight was lessened by twelve pounds.

Further observations in healthy men with these impure and complex substances would be merely repetitions of the above and are consequently omitted, for nothing would be emphasized by them except the general variability of the toxic properties of all of these dried preparations of thyroid.

### C. *Thyroidism in diseased man.*

So far my observations as to the production of "thyroidism" by the thyroid material have included experiences only upon healthy men and other healthy animals. In reality, however, observation 1 of the following series first drew my personal attention to this subject. The remaining experiences that are described here are still more apropos.

Obs. 1. Adult man, 55 years of age, with paralysis agitans, took daily except Sundays about 8 grm. of finely minced fresh sheep's thyroid for a period of three weeks. The effect was absolutely negative. After one week had elapsed two 5-grain sugar-coated thyroid tablets of B. W. & Co. were given three times a day. On the morning of the third day the

following symptoms of intoxication had developed: Excessive restlessness during the preceding night; severe dizziness and a sensation of tension over the forehead; severe dyspnœa; strong but irregular cardiac action, pulse 120 per minute, and temperature  $103\frac{1}{2}$ ° F. The thirst was insatiable, the general prostration considerable, and the face and neck were covered with a very prominent fiery red erythematous rash. In the afternoon of the same day a slight diarrhoea developed. The tablets having been discontinued, all these symptoms disappeared in the course of a few days and the patient rapidly gained in flesh and strength.

Obs. 2. A girl aged 18 years, with exophthalmic goitre. Pulse 125; temperature 99° F. Circumference of neck  $13\frac{1}{2}$  inches. Left lobe of thyroid larger than right, and right eye more prominent than left. Tremor, insomnia, and numerous other symptoms including cramps and occasional attacks of tetany. Took for the first six days 6 grm. of fresh sheep's thyroid slightly cooked. Result, pulse 120, temperature 99°. Circumference of neck 13 inches (I had been previously informed by the patient that the struma often varied in size). 10 grm. of the finely minced raw gland were then given daily for twelve doses, after which the pulse was 110, the temperature  $98\frac{1}{2}$ ° F., and the measurement of the neck 12 inches. The weight of the patient had increased one pound. Previously to taking the fresh raw material this girl had been given one tablet (same lot as in Obs. 1) twice a day for three days, with the result that the tremor, cardiac symptoms, and general weakness decidedly increased. The left eye became more prominent and the habitual diarrhoeic movements that usually occurred in the afternoon appeared in the morning also.

Obs. 3. Exophthalmic goitre, but with no evident enlargement of the thyroid gland. Pulse 136, temperature  $98\frac{2}{5}$ °, weight 125 pounds. Took for one week except Sunday 10 grm. per day of fresh sheep's thyroid. Result, pulse 120, temperature  $98\frac{2}{5}$ ° F., weight 126 pounds. This man had been troubled with almost daily sudden attacks of diarrhoea previously to the beginning of the course of thyroid, and, therefore, for the first few days capsules of pepsin and subgallate of bismuth were given after each meal, the thyroid dose being taken three hours after breakfast. Under this treatment the diarrhoea quickly disappeared and did not reappear during the remainder of the time that the fresh gland was taken.

Obs. 4. Exophthalmic goitre; female, aged 28 years. Previously to coming under my charge she had taken fresh pigs' thyroid for a number of weeks in daily doses (amount unknown). After eight or ten weeks of this treatment in combination with baths and careful dieting she had gained several pounds in weight. The goitre had diminished somewhat

at first, but later enlarged to its former size on stopping the treatment; no increase in the pulse rate or palpitation had been noticed. Some months later she had been given capsules of one of the dried American thyroid preparations. From the use of this preparation such a considerable loss in flesh, severe diarrhoea, marked increase in the general weakness, and severe tachycardia occurred that the preparation had to be discontinued.

This patient was given the perfectly fresh raw thymus (about 4 ounces) daily for a number of months with the result that the pulse rate decreased from 100 per minute to 84. The enlarged and firm thyroid became much softer, and diminished greatly (2 in.) in size. The absent menses returned. After a few doses of the pepsin and subgallate of bismuth the troublesome diarrhoea permanently disappeared; the exophthalmos was greatly lessened and von Graefe's sign entirely gone. The weight increased from 105 lbs. to 125 lbs. under this nutritious diet, and the strength is now such that the patient can walk four or five miles per day without fatigue.

Obs. 5. A man aged 20 years, with mild Graves' disease, had been taking twelve 5-grain thymus tablets of B. W. & Co. daily for two weeks. Without my knowledge he concluded to double the dose, and in three days, in addition to great thirst, dizziness, great restlessness, unsteadiness in gait, considerable dyspnoea and precordial oppression were developed. Accompanying the above were nausea, vomiting, diarrhoea and very severe general prostration. The face, arms and neck were covered with large urticarial wheals. The temperature rose to 102° F., the pulse to 150. The tablets were immediately stopped and most of the above disagreeable symptoms disappeared by the following morning, although the general depression and diarrhoea continued for several days. With very much larger doses of the fresh thymus no such symptoms reappeared in this case.

In one other instance I have seen the above symptoms occur during the administration of these thymus tablets even in doses of eighteen per day.

Obs. 6. Finally, I have had the opportunity of carefully examining and questioning an intelligent patient with myxedema who took for about two months small doses of a certain commercial dried preparation without the least benefit; in fact, according to her statement, the various symptoms of the disease became more marked. The preparation in question produced in this case frequent attacks of severe diarrhoea, considerable acceleration of the pulse rate, headache, and much prostration. Another commercial preparation was substituted, and in consequence the

myxoedematous symptoms rapidly improved, while the diarrhoea, headache, etc., disappeared and did not reappear unless large doses of this latter preparation were taken daily for several days. It should be noted that the doses of the respective preparations were the same, in this instance each tablet being equivalent to 5 grains of the raw gland.

About 30 grammes of the former preparation being in my possession, ten tablets were given daily to a thyroidless dog before the cachexia had developed. The appearance of the latter was certainly not delayed, on the contrary the dyspnoea and fits presented themselves earlier than usual (about 38 hours). On injecting an extract of these tablets into another thyroidless dog the cachexia appeared in 30 hours, and none of the symptoms lessened in severity from even repeated injections of this extract. The preparation undoubtedly contained very toxic substances, but whether any of the supposed active curative thyroid principle was present cannot be definitely stated, owing to the fact that the failure to relieve the symptoms in the above tests is not conclusive proof of its total absence. The important objections to these tests will be discussed more fully in Part III.

#### D. *Results and general considerations.*

The results of the foregoing observations conclusively demonstrate the two following main facts:

First, that the ingestion of very large amounts of finely minced fresh thyroid material gives rise to no intoxication whatever in man, monkeys, dogs, cats, rabbits and birds, provided the digestive processes in the said animals be performed with the usual normal rapidity.

Second, that the stale material or the preparations that have been made from the thyroid according to the methods usually in vogue may or may not produce in certain animals the symptoms of an intoxication. In many instances the symptoms may follow even comparatively small doses.

In normal men, monkeys, dogs, cats, rabbits and birds I have never been able to produce by means of the fresh thyroid material a rise of temperature, acceleration of the pulse either shortly after the ingestion of the dose or on the following day, loss in weight except in rabbits (?), agitation, general prostration, or exophthalmos. Further, none of the above normal individuals exhibited any perceptible enlargement of the thyroid gland, nor did accurate comparative measurements of the lobes

before and after the ingestion of the thyroid indicate that any discernible diminution in size had occurred.

Carnivorous animals (dogs and cats) and birds (cocks) appear to be insusceptible to the action of the toxic preparations even in enormous doses; but man, monkeys, and especially rabbits, speedily manifest the various symptoms in greater or less prominence. This marked difference was not surprising in the least, for many previous investigators of poisonous meat, sausage, cheese, and other ptomaine-containing food-stuffs, note the frequent immunity of carnivorous animals from these poisons. The natural propensity of cats, and of dogs in particular, for all kinds of carrion and sundry bits of putrefied food is also well known and needs no comment further than the remark that no ill effects follow numerous meals of this character. Boehm\* reports that a dog was given a large bowl full of poisonous cheese, all of which he ate, but no toxic symptoms followed. Chickens and sparrows, according to Gaertner,† are also insusceptible to toxic meat, and other observers have reached a similar conclusion. In dogs, however, as has been stated elsewhere, Lanz noticed some acceleration of the pulse rate, but Edmunds ‡ produced no effect by feeding dogs on sheep's thyroids.

All the dogs and other animals to which I gave the dried commercial thyroid preparations exhibited great thirst, but as these preparations are often one-half chloride of sodium, considerable thirst is what one should expect. Great thirst consequently indicates nothing that could be definitely attributed to the special action of the toxins. As regards the emaciation noticed by Lanz I may add that at the beginning of the experiment the majority of my dogs were very thin and poorly nourished, but while taking large quantities of all kinds of thyroid material and receiving excellent food, they rapidly fattened and continued to do so while under observation. If these animals had been kept housed for a number of months and fed on thyroid they naturally would have ultimately fallen into a poor condition; and so would other dogs if deprived of out-door exercise and air, no matter

\* Boehm, Ziemssen's *Handbuch*, Bd. xv, p. 249.

† Gaertner quoted by Oestertag, *Fleischbeschau*. Stuttgart, 1892.

‡ Edmunds, loc. cit.

how well fed the animals may be. Normal carnivorous animals, in fact, are not at all suitable subjects for such experiments if one has to keep them in confinement for a long time. Most of the toxic thyroid preparations do cause considerable loss in weight in rabbits, monkeys, and in man. It is conceivable that the fresh material may do this also if gastro-intestinal fermentation occur and the altered thyroid products become absorbed. This latter circumstance, I think, very probably explains many of the experiences in which clinicians have observed mild or severe toxic symptoms to follow the ingestion of fresh raw material. This marked katabolic effect of the substances contained in these toxic preparations is not, however, specific for the thyroid only. Preparations of other ductless glands are liable to produce a very similar intoxication accompanied by emaciation. Many tox-albumoses, toxic proteids and ptomaines from poisonous meat and sausage, fish, cheese and other food-stuffs, usually produce a similar marked emaciation, as is clearly shown in the well-known works of Bollinger, Brieger, Backlisch, Müller, Panum, Marino-Zucco, and many other observers.

Generally speaking the intoxications produced by all of these poisons from altered food-stuffs resemble one another in some respects, that is, certain symptoms appear to be more or less common to them all, while other symptoms are often essentially different. My experiences with the toxic thyroid material teach that this statement is valid for the clinical picture of this variety of intoxication also. Neither in the acute nor in the chronic forms of "thyroidism" are the various individual symptoms always uniform as a whole. Thus one commercial preparation seems to affect the gastro-intestinal canal chiefly; another preparation, even from the same firm, appears to suddenly overwhelm the muscles; others act upon the central nervous system of monkeys and of man also, so that either a spastic or a flaccid form of motor paresis or of paralysis may occur. Sometimes exophthalmos is prominent; at others no such condition is visible. All of these toxic substances seem to profoundly alter the blood.

In monkeys the majority of thyroid preparations produce a rise of temperature; on the other hand some of them in concentrated doses

produce just the opposite effect, a fall of 4° F. below the normal having been witnessed by me. In some cases dyspnæic symptoms are marked; with other preparations the respiratory rhythm may from the beginning become progressively slower and finally cease, the excessively rapid heart-beat continuing for a short time afterward. In some of my monkeys no tremor or restlessness was noticeable; several others, however, exhibited this agitation very prominently. Acceleration of the pulse appeared to be produced by all the toxic thyroid preparations that were tested by me.

An analysis of these contradictory physiological effects appears to indicate that very probably these toxic preparations contain frequently different toxins or at times a combination of toxic substances. In certain preparations one variety of toxin or toxins predominates, in others a toxin of a slightly different nature may occur and superadd its effects to those produced by the other toxins which possibly enter into the composition of the preparations in question.

From Observation 6 under C, and from practically similar ones of other clinical observers, I am warranted also in stating that the toxic action of some of these dried thyroid preparations exceeds their palliative action in myxœdema; in fact certain symptoms of the latter disease may be made worse thereby, and no palliation at all as regards certain others may follow. This fact does not, however, necessarily prove that none of the supposed thyroid principle, which most observers, as far as I am aware, concede to be present in the thyroid gland, is contained in the preparation; for until this principle is obtained in a pure state, the above clinical test of the complex crude product only demonstrates that either the susceptibility of the individual is very great or that the toxins are particularly active, or both. If a certain toxin be present in considerable amount, this very toxin may possibly nullify or partly counteract any effect resulting from the beneficial thyroid principle or principles.

In reality many of the symptoms produced by these toxins from the thyroid material and from other toxic animal material also, show a remarkable and suggestive similarity to those composing the clinical picture of the experimental acute cachexia thyreopriva. Many of

the symptoms that are exhibited by intoxicated men, monkeys and rabbits on the one hand, and by thyroidless carnivora in the early stages of the acute cachexia on the other, bear also a striking similarity to some of the most prominent symptoms and complications of exophthalmic goitre. Thus in all three conditions the central nervous system manifests its over-activity by fibrillary muscular tremor or twitching, tetany, etc. The contractions of individual muscles or groups of muscles so common in the intoxications and in the cachexia, frequently occur also in exophthalmic goitre. Rigidity, contracture, and a variety of spastic paralysis (Marie, Chareot) are also symptoms which may be included in the symptomatology of each. The rapid emaciation, the prostration and the weakness occurring with the intoxications, with the acute cachexia, and usually with Graves' disease, need no comment. A rise of temperature is frequent in exophthalmic goitre, is usual in the intoxications; and on the first few days following the thyroideectomy, during the acme of the muscular twitchings in the cachexia, the temperature often rises  $4^{\circ}$  or  $5^{\circ}$  above the normal (Hertzen, Ughetti, Alonzo, Rogowitsch, Horsley). Considerable acceleration of the pulse is also invariable, I find, in the early stages of the acute cachexia in dogs and monkeys. Slight exophthalmos \* in the experimental cachexia is rarer, but does occur under circumstances which I shall describe in the following division of this paper. Some of the toxins from stale meat, thymus, thyroid, etc., frequently produce this symptom to a marked degree in rabbits and in monkeys, although in normal dogs I have utterly failed to produce this effect when the toxic substances were given by the stomach.

In exophthalmic goitre my own rather limited experiences and the very numerous clinical reports of others clearly indicate that certain symptoms are very materially increased by these toxin-containing thyroid preparations, whereas the absolutely fresh raw material produces, according to my experience, no such result.

The advocates of the theory that a too active thyroid produces Graves' disease, while admitting that possibly some of the symptoms

\* Edmunds has also noticed the occurrence of exophthalmos after removal of the thyroid.

following the ingestion of the toxic thyroid preparations may be due to chance ptomaines, may raise the objection that the supposed thyroid principle is of the nature of an enzyme (White and Davies, Notkine), and that the thyroid gland contains immediately after its removal the zymogen only of this enzyme. This state of affairs is well known to exist in the case of the pancreas, which after removal develops digestive properties only after a certain period of time has elapsed. The nature of this generally conceded thyroid principle is, however, very obscure, but were this enzyme the active principle, toxic in character, one might reasonably suppose that it would under varying circumstances preserve its toxicity, that is to say, if this special substance be obtained in a pure form it should continue to be toxic as before, though possibly the toxic symptoms that it produces might differ somewhat from those produced by the crude toxic material. I have consequently endeavored (Part III) to isolate from the various preparations of thyroid sundry substances in a pure state which have been given to normal and also to totally thyroidless or partially thyroidless animals. It is needless to say that the results in this direction do not in the least disagree with those obtained in the present part of this paper.

I reiterate that the fresh raw thyroid material shows not the least tendency to produce intoxication; that I have not found that the symptoms of exophthalmic goitre are increased by its ingestion, providing of course that no toxins develop in it during gastro-intestinal digestion; that a certain toxic substance or group of substances may or may not be present in the stale thyroid material and other animal material, which substances may produce in certain animals symptoms somewhat analogous to many of those usually met with in exophthalmic goitre. Though both experimental and clinical evidence demonstrates the fact that these analogous symptoms are produced by the toxic thyroid preparations, this fact cannot be logically or justly brought forward even as auxiliary proof to support the theory of those (Moebius and others) claiming that an over-active thyroid is responsible for the various symptoms manifested in Graves' disease.

PART II. THE INFLUENCE OF FOOD UPON THE APPEARANCE OF THE ACUTE  
CACHEXIA THYROIDECTOMICA.

A. *Historical introduction.*

Munk \* having observed that a diet composed of lumps of meat hastened the death of thyroidless dogs. Breisacher,† a pupil of Munk, investigated this relationship and found that a certain number of thyroidless or partially thyroidless animals could be kept alive if fed with milk or with milk and eggs only. If raw meat or soup were given to these apparently normal animals the characteristic symptoms of the cachexia quickly developed. Meat carefully extracted by long boiling did not produce this effect. In animals in whom  $\frac{1}{5}$  or  $\frac{1}{4}$  of the thyroid had been left the bouillon was well borne, but as soon as the remaining portion of the gland was removed the symptoms of the cachexia quickly appeared, providing the raw meat or bouillon were given. If the meat salts were mixed with the milk the action was occasionally similar to that of the bouillon. In certain thyroidless dogs, although the tonic and clonic muscular contractions and dyspneic attacks had appeared, these symptoms disappeared if the milk diet was continued. De Quervain,‡ and later Rosenblatt,§ also conclude that the progress of the cachexia is milder if the animals are fed on milk only. Masoin,|| on the other hand, claims that this pretended influence of milk diet upon the cachexia does not exist and that the duty of the thyroid gland is to nullify various toxins that are produced by the organism, which toxins accumulate in the blood and give rise to the convulsions, etc. The antitoxic function of the thyroid, previously emphasized by Colzi, Rogowitsch, Fano and Zanda, Schiff, Gley, and many others, is insisted upon by Masoin, and the intoxication is thus classed with the auto-intoxications.

The experimental evidence as to the influence of different food-stuffs, consequently, is by no means clear, and, as Ewald has remarked apropos of Breisacher's results, it is by no means definitely settled whether it is the disturbance of the metabolic processes in the tissues of thyroidless animals which directly produces the cachexia, or whether it is the components of various food-stuffs which are directly toxic. The mere administration of various kinds of food does not, as Ewald states, definitely settle this question, but from such experiments one can gain a fairly definite knowl-

\* Munk, *Berl. klin. Wochenschr.*, 1889.

† Breisacher, *Arch. f. Anat. u. Physiol.*, Physiol. Abtheil., 1890, 509.

‡ de Quervain, *Virchow's Archiv*, cxxx (1893), 504.

§ Rosenblatt, *Arch. d. scien. biolog.*, St. Petersburg, iii, No. 1, 1894.

|| Masoin, Paul, *Arch. de phys. norm. et path.*, 5 sér. vi (1894), 291.

edge of the relative susceptibility of certain animals before and after thyroidectomy to the constituents of certain foods, provided certain factors, which if present are liable to give rise to very erroneous conclusions, be duly taken into consideration.

Briefly summed up, these factors which so seriously complicate the study of this problem are nothing more than the entire series of those "conditioning circumstances" (Horsley) upon which, as Horsley and others point out, the effects of thyroidectomy depend. Thus of all classes of animals the thyroidectomized carnivora, dogs, cats and foxes, exhibit the severest and most rapidly developed cachexia, death usually occurring between the third and fourth day in a comatose state (Wagner). According to Horsley the average duration of the neurotic, or first stage of the cachexia, is from one to two weeks in dogs; from one to three weeks in monkeys; young dogs and young monkeys alike dying in this stage. Elderly dogs merely exhibit symptoms of indolence and incapacity, indicating that the activity of the gland appears to be most active when tissue metabolism is most active. Under "other conditioning circumstances" Horsley \* further dwells upon the important influence of a state of low nutrition before thyroidectomy, and upon the influence of the surrounding temperature upon thyroidless animals.

Possible residual thyroid tissue and the existence of accessory glands (Piana, Wagner, Fuhr, Ewald, Carle, Autokratow, Gley and Phisalix) and para-thyroids (Sandstroem, Baber, Gley, Kohn, Edmunds), may exert so great an influence that either the cachexia may not appear at all in dogs, or after a few very mild symptoms have been exhibited all signs of it may entirely disappear.

The operation of removing the thyroid lobes of the dog is an exceedingly simple one, but if the lobes be shelled from their capsules, as is usually done, one or other of the small para-thyroid bodies may be, as Gley has pointed out, unintentionally left behind. Should two or more be left, very slight or even none of the symptoms of the cachexia may sometimes occur, a personal experience which agrees with that of Gley. In certain young dogs both thyroid lobes, the para-thyroid bodies and any neighboring discernible accessory glands may be removed, nevertheless the results may be the same, or after the expiration of from five to ten days the severer nervous accompaniments of cachexia may develop, frequently with astonishing rapidity. In the first convulsive attack the dog may die, or several attacks of dyspnoea, convulsions, etc., which progressively increase in severity, may succeed one another during the

\* Horsley, loc. cit.

next few days, the dogs ultimately dying often during a severe convulsive attack. Other dogs and also cats may exhibit only moderate fibrillary twitchings and stiffness of the hind legs for numbers of weeks or even months, becoming to all appearances myxœdematous, and finally die in that condition. In a few others, after slight symptoms have existed for a number of weeks, they apparently subside completely and the dogs seem normal. The two latter courses, it is almost needless to remark, are generally exceptional.

According to my experience the vast majority of young dogs in good condition will, when they are fed on ordinary raw meat both before and after the operation, begin to manifest the symptoms of an impending dyspnoëic attack within 52 to 54 hours after the total excision of the thyroid, and usually die in convulsions very shortly after the dyspnoëic attacks have begun. In such dogs I consider that it may be justly assumed that for all practical purposes a total thyroidectomy had been performed. Very evidently none of the accessory glands that possibly remained were sufficiently active for the performance of much of the function of the thyroid. Nevertheless a careful dissection of the hyoid and aortic regions of some of the apparently thyroidless dogs, and of a large number of normal dogs also, frequently resulted in my finding one or more tiny, pale reddish globules from 5 to 1 mm. in diameter, which under the microscope presented a structure identical with that of the para-thyroid body, or parts of a continuous section appeared similar to that body (Plate XV, Fig. 2), while other fields of the same section had the appearance of the true thyroid structure. In all my young dogs and also in two cats, in which the cachexia developed mildly and not until four or five days after the operation, these tiny accessory bodies were invariably found. In some of the dogs additional accessory bodies of greater dimensions (2 to 8 mm.), such as Fuhr, Piana and others describe, occasionally accompanied the smaller ones. Two dogs that survived for sixteen days possessed each three small aortic bodies only about 1 mm. each in diameter, and in both dogs I found on the right side of the root of the neck just above the sternum a small round accessory body about 1.5 mm. in diameter, similar in structure in every way to the normal thyroid body.

The minuter aortic accessory bodies, often embedded in fat, are exceedingly difficult to find, and during a careful dissection by even the most experienced are very liable to be passed aside as bits of fat. Small bodies, apparently thymus remains, will also be met with and macroscopically may easily mislead. My own plan was to trust only to the microscope and examine everything that appeared to be the least suspicious. In

numbers of instances where this latter plan has been neglected by observers and judgment pronounced as to the absolute absence of those accessory bodies, I feel very confident that many erroneous statements have been made.\* Too much stress, therefore, cannot in my opinion be laid on the importance of this factor in influencing both the rapidity of the appearance and the degree of the cachexia in dogs and cats.

It is easily seen that in consequence of this latter complicating conditioning circumstance, numerous experiments, many careful and tedious dissections, and much laborious microscopic examination of suspected bits of tissue had to be performed before I could obtain a really definite idea as to the average number of hours which usually elapsed between the operation and the beginning of the dyspneic attack in the totally thyroidless dog. The average that was given above was obtained from fifteen young dogs of about six months to one year in age, and the animals were fed both before and after the thyroidectomy on raw meat.

In studying this problem naturally the contents of the intestine at the time of the operation had to be regarded as a possible source of origin for sundry ptomaines, etc., whose additional action might complicate the results. Every operation was thus performed not earlier than twenty-four hours after the last meal, and in addition precautions were taken with a number of animals to feed them on bread and water for at least two days before performing the thyroidectomy. This latter precaution, I may add, did not, however, appear to influence the average time of development one way or the other. But if the dogs were fed on bread and water alone for several weeks before the operation the general condition of the dogs was liable to become so poor that the cachexia frequently developed rather more rapidly. A preliminary diet containing a greater amount of albuminous food is thus to be preferred.

My experience with totally thyroidless monkeys has not been large, but within twenty-four hours after the operation I have noticed, even in partially thyroidectomized monkeys, moderate fibrillary muscular twitchings, sluggishness, and a very marked increase in the susceptibility to cold; the animals constantly keeping on the day after the operation close to the steam heating pipes, while before the operation no proclivity for the hot pipes had been exhibited by the monkeys, which were kept in a room always carefully maintained at a uniform temperature.

\* In herbivorous animals also (calf and lamb) a number of these small bodies are usually present, and possibly their presence accounts in a great measure for the long period of time required by these animals to fully develop the symptoms of the cachexia.

My general mode of procedure was essentially the same as has been pursued by Breisacher and others and needs no further comment.

Comparative observations were thus made in which perfectly fresh raw meat, extracted meat and special extracts from fresh and from stale meat were used. Fresh and also dried toxic thyroid material were given to certain animals and the resultant effects noted. Comparative experiments with undoubtedly fresh milk were naturally out of the question in a large city to which the milk is brought often from a distance of many miles.

### B. Experiments.

1. *Dogs.* In the following table (p. 192) I have tabulated certain of my trustworthy experiments on the dog in the form of series. In each series I report with three exceptions only those animals in which not the slightest trace of residual thyroid, para-thyroid, or accessory thyroid tissues could be found after a most careful dissection and a microscopic examination of all tiny bits of possibly suspicious tissue. In series XIII, Dog 3, I was inclined at first to question the actual absence of one or more of the minuter accessory bodies, but later experiments in which certain thymus extractives were injected lead me to attribute the short delay of the dyspnoea to the effects of the thymus diet.

Necessarily from the nature of the experiments the figures are but approximate, indicating in a general way the average number of hours that elapse after total thyroidectomy before the average young dog in an average condition begins to manifest the dyspnoeic attack.

Numerous necessary exclusions have unfortunately caused the number of trustworthy observations in some of the series to diminish considerably, and in consequence it may be objected that some of the averages include too small a number of definite uncomplicated observations, especially as the averages differ not by days but by hours only. Granting the validity of this objection and also the possibility that the actual figures of some of the averages of the series might be slightly different had not so many of my dogs (60 per cent.) been provided with accessory bodies, I nevertheless offer these results for what they are worth.

*Experimental Thyroidism*

INFLUENCE OF FOOD UPON THYROIDECTOMIZED DOGS.

DIET.		Before operation.	After operation.	Days since beginning.	Number of days passed.	Resid. of access.	Resid. of thyroid.	Number of observations.	Max. hours before dyspnoea.	Min. hours before dyspnoea.	Average time of series in hours.	Remarks.
Number of dog.	Number of dog.											
I.	1	Raw meat.	Raw meat.	52	53	None.	None.	15	56	47	53	Died in second fit.
II.	3	Bread.	Raw meat.	51	52	None.	Small aortic.	6	54	49	52	
III.	4	Bread.	Milk.	48	54	Small aortic.	None.	4	58	48	53	Severe diarrhea.
IV.	4	Milk.	Milk.	50	58	None.	None.	8	60	45	53	Poor milk for No. 8.
V.	2	Milk.	Milk.	45	48	None.	None.	6	78	52	65	
VI.	2	Bread.	Bread.	64	69	None.	None.	6	60	52	56	
VII.	1	Bread.	Boiled meat.	52	56	None.	None.	3	70	53	64	Died in first fit.
VIII.	2	Bread.	{ Fresh raw meat.	70	71	None.	None.	3	76	58	65	
IX.	1	Raw meat.	Bread.	58	66	None.	None.	3	65	58	63	
X.	1	Raw meat.	{ Fresh raw meat.	65	72	None.	None.	3	65	58	63	
XI.	4	Raw meat { and dried thyroid.	Bread and dried thyroid.	38	42	None.	None.	8	54	38	43	Diarrhea in nearly all these dogs.
XII.	2	Raw meat.	{ Fresh raw thyroid.	96	113	{ One small aortic.	4	96	58	68	No. 4 also repeatedly injected with thyroid after beginning of fits.	
XIII.	3	Raw meat.	Bread&raw thymus.	92	97	None.	3	92	60	76		
XIV.	4	Raw meat.	{ Stale raw thyroid.	88	96	None.	3	88	62	75		
XV.	3	Boiled meat.	{ Meat bouill. &ion&bread.	45	47	None.	4	53	45	48		
XVI.	1	Raw meat.	Milk.	54	60	None.	3	56	47	52	No autopsy on dog 1. Milk of excellent quality.	
XVII.	2	Raw meat.	Meat stew.	49	54	.....	2	50	49	50	Spleen removed 23 days previously.	
XVIII.	1	Raw meat.	{ Fresh thyroid, boiled meat.	96	125	Three aortic.	2	....	53	53	Acceleration of pulse, elevation of temperature, and emaciation occurred in all these dogs. The wounds remained aseptic.	
				58	66	None.	2	58	53	56		

Disregarding the diet of the animals previous to the operation and averaging the various series, one finds that the influence of the after-diet appears to be as follows: raw meat, 53 hours; fresh raw meat, 63 hours 30 minutes; bread and soup, 52 hours; ordinary meat extracted by boiling, 56 hours; city milk (fair quality), 52 hours; wheat bread, 65 hours; meat stew and fresh raw thyroid, 56 hours; fresh thymus, 75 hours; toxic thyroid material, 45 hours 30 minutes; fresh thyroid, 76 hours.

These averages would appear at first sight to indicate that both the kind and the quality of the diet do to a very slight degree influence the average time of appearance of the dyspnœic attacks in totally thyroidless dogs, but the differences are entirely too small in my estimation for one to positively assert the absolute correctness of the indication. The differences are certainly not as great as the observations of Breisacher would lead one to expect, practically no difference between the effect of ordinary raw meat and that of milk appearing to exist in the totally thyroidless dogs. In fact no matter what the diet may be, the totally thyroidless young dog very quickly develops one or more of the symptoms of the cachexia and dies usually within a few days after the performance of the thyroideectomy. Even if large amounts of fresh thyroid material be included in the diet the appearance of the cachexia is evidently but slightly delayed, although some of the symptoms are frequently modified remarkably in severity. Nevertheless I have not been able by this means to prolong the lives of totally thyroidless young dogs longer than 97 hours after the thyroideectomy. Possibly this period might have been extended had the minced thyroid been successfully introduced into the stomachs of the dogs by artificial means, for I have noticed that generally by the second or the third day food of all kinds is usually left untouched, water alone being voluntarily swallowed in small quantities. On attempting to feed the dogs through the stomach tube, even after cocaineizing the fauces, it was found that, although very small amounts of milk and thyroid had been injected, practically the whole amount was almost immediately vomited. Under these circumstances and with the rapidly progressive emaciation and general weakness, death of course quickly followed.

If the totally thyroidless dogs were fed with large quantities of fresh

thymus gland or with concentrated bouillon from the fresh gland, a somewhat similar though less marked effect both upon the time of appearance of the dyspnoea and upon the severity of the symptoms in general appeared to occur. In an earlier series of experiments, to which I have referred elsewhere,\* I did not notice this favorable influence of the thymus when fed to the thyroidless dogs; but on repeating the earlier experiments and using much larger quantities of either the fresh thymus or of the concentrated thymus bouillon, I got the very appreciable beneficial effects to which I now call attention.

The dried commercial thyroid preparations and dried thyroid prepared by myself did not appear to delay the cachexia in the least, even though the doses of the dried thyroid were increased to 30 grammes (equivalent to about 100 grammes of the raw thyroid) per day. Judging from the average of the figures obtained in the eight dogs mentioned in the table, the ingestion of these preparations seemed on the contrary to hasten slightly the severer symptoms of the cachexia. It should be stated, however, that small portions of all of the dried preparations used in this series of dogs had been given to normal rabbits or monkeys and all had proved very toxic to both the latter animals. Two of the preparations contained the active palliative thyroid substance also, for by chemical means the substances that appeared to be toxic when taken by the mouth were removed, and the active palliative substances were injected subcutaneously into totally thyroidless dogs with very favorable results.

According to my experience, the cachexia so quickly overwhelms the completely thyroidless young dog, whether fed on milk, bread, or even fresh thyroid, that the comparative effects of different foods cannot be accurately tested upon the same dog, and the plan pursued by Breisacher of partial thyroidectomy would appear to be the one to be followed if the dog is to be the animal chosen for the experiment. Although it is open to many serious objections, this method was employed in eight dogs, but I got nothing that could be called an unquestionably positive result. The method was as follows: In seven young anaesthetized dogs the whole of the right thyroid along with its capsule was removed. On the left side

\* *Med. Record*, New York, June 15, 1895.

about the upper fourth or fifth was left, none of the large superior vessels being interfered with. In the eighth dog the left lower fourth only of the thyroid body was left, precautions being taken to disturb its vascular supply as little as possible.

Four of these partially thyroidless dogs did not appear to differ in the least from normal dogs, and from the second day after the operation were fed on raw meat for three to four weeks. During this period no symptoms of the cachexia were manifested. Of the remaining four, the three in which about the upper fifth of the left lobe had been left presented evident though slight tremor in the rear extremities by the second day, and in running about appeared to use the hind legs somewhat clumsily. On the third morning these symptoms could not be detected and two of these dogs were put upon a diet of stale but good meat. As no further symptoms occurred, after two weeks of this diet, the two dogs were used for other experiments. To the third dog were given, besides his bread and water diet, 20 grm. of a dried commercial thyroid preparation, 1 grm. of which had proved fatal to a rabbit. On the next morning the temperature of this dog had risen  $1^{\circ}$  F. and the pulse rate had increased by thirty beats. In the hind legs the tremors had reappeared and a slight degree of stiffness and awkwardness in gait was apparent. Having but 10 grm. left of this thyroid preparation, this amount was administered and the dog carefully examined on the following morning. The temperature and the pulse were the same as on the previous day and the tremor and tetany were also still prominent. The bread diet was changed to raw meat and the dog observed for three weeks. On the seventh day the temperature and pulse were the same as before the operation, and during the rest of the time the animal did not appear to differ from a normal dog.

The dog in which the lower fourth of the left thyroid lobe was left presented by the third day considerable tremor, tetany and emaciation. On the fifth day the dog appeared better, took a little bread and a small amount of thoroughly boiled milk. On the ninth day (afternoon) the wound was found perfectly healed, and but for a just perceptible occasional fibrillary twitching in some of the muscles of the hind extremities the animal did not seem to differ from a normal one. On the tenth morning it was found in a fit from which it recovered in a few minutes, exhibiting, however, the rapid respiration and dyspnoea. After three similar fits during the day and probably more during the night the animal died on the eleventh day, in spite of several large subcutaneous doses of a commercial thyroid elixir. On dissecting out the remnant of residual gland it was found to have undergone almost complete atrophy, due

doubtless to insufficient blood supply. The residual thyroid in the preceding seven dogs had on the contrary become considerably hypertrophied, the blood supply apparently not having been interfered with.

In four of the dogs, therefore, not enough of the thyroid had been removed to even temporarily disturb the physiological equilibrium. In two of the remaining dogs it was evident that though some disturbance had followed the partial excision of the gland, the vulnerability of the two animals was not sufficiently diminished to show the possible effects resulting from stale meat diet. A slight susceptibility to the toxic substances contained in the dry thyroid preparation would appear to have been produced in the seventh dog, but one cannot positively assert that the slight temporary return of the symptoms was not simply a coincidence dependent upon a temporary disturbance of the activity of the residual thyroid tissue. At any rate it was evident that the functional activity of the residual thyroid had been sufficiently recovered by the seventh day for the metabolism of this dog to proceed in a practically normal manner.

In the eighth dog, though the progress of the cachexia was slower, the final effect was the same as that following total thyroideectomy. Thus, in spite of the bread and milk diet the fits occurred and the cachexia progressed just as one frequently sees it in dogs in which both thyroid lobes have been removed, but in which a certain amount of accessory thyroid tissue has been unavoidably left.

It is well known that it is extremely common for dogs in which a small amount of residual or accessory thyroid tissue has been left, to have sundry dyspnœic and convulsive attacks, between which the dogs may seem normal. Consequently, if the milk diet is changed to one of raw meat, and the dogs, like those of Breisacher, have an attack after one or two days, it is absolutely impossible to decide whether the ingested constituents of the meat really induced the attack or not, for it is readily conceivable that the dogs might have exhibited the symptoms had no meat at all been given. One very definite fact obtained, however, is that the cachexia develops in the totally thyroidless dog just as readily with the milk diet as with the meat diet.

2. *Monkeys.* In monkeys the results appeared to be slightly more definite, for shortly after the partial thyroideectomy these animals appeared to become more sensitive to moderate doses of certain commercial meat extracts, as the following experiment indicates.

A large normal Java monkey was given 35 grm. of a commercial meat

extract. This produced a moderate diarrhoea which disappeared in twenty-four hours, but no other disturbance could be detected. On the sixth day 20 grm. of the same boiled meat extract were given, but on this occasion no diarrhoea resulted. After the expiration of two days the dose of 20 grm. was repeated and was again well borne by the monkey.

Partial thyroidectomy was then performed, approximately the upper fourth of the left thyroid lobe being left, and the monkey confined to a diet of bread, fruit and water. On the morning after the operation occasional fibrillary twitchings were distinctly present in many muscles, the temperature had risen from  $101\frac{2}{3}^{\circ}$  F. to  $103^{\circ}$  F., and the heart-beat from 100 to 156. On the second day the monkey appeared to be chilly and continually kept close to the steam heating apparatus and could not be enticed away from it even by a banana, of which the monkey was very fond. Pulse 186; temp.  $104^{\circ}$  F. The aseptic dressing was removed and, after the small wound had been found to be in perfect condition, was replaced.

By the fifth day the appearance of chilliness had disappeared, the temperature had become  $102^{\circ}$  F. and the pulse 112. On the ninth day the wound was re-examined and found to be completely healed. The temperature was  $99\frac{2}{3}^{\circ}$  F., the pulse 96, and the monkey seemed as lively as before the operation. On the tenth morning 15 grm. of a meat extract concentrated from perfectly fresh ox muscle was given to this monkey, but without affecting the animal in the least.

Two days later at 11.30 A. M. 10 grm. of commercial meat extract, the same kind that had been given to the monkey before the partial thyroidectomy, which had been sterilized by boiling and kept in a sealed tube from the day on which the original package was opened, was administered by means of the stomach tube and the following effects resulted: After the expiration of about one hour the monkey became quite restless and had one large watery movement. In about two hours and a half the animal exhibited still greater restlessness, and the temperature had risen from  $99\frac{2}{3}^{\circ}$  F. to  $101^{\circ}$  F., and the heart-beat to 140 per minute. At this time a slight though easily discernible degree of exophthalmos accompanied by horizontal nystagmus was apparent. By six o'clock in the afternoon a considerable degree of fibrillary tremor was present, but the transient exophthalmos and nystagmus had almost disappeared. On the following morning the temperature was  $103^{\circ}$  F., the pulse 180 per minute; the fibrillary tremor still marked, but the exophthalmos and nystagmus had entirely disappeared. The animal appeared to be again chilly, and when removed from the neighborhood of the steam pipes it immediately returned, exhibiting considerable awkwardness and stiffness in its move-

ments. Throughout the day the thirst was very pronounced, although the diarrhoea had practically disappeared. On the afternoon of the second day the pulse was 100, the temperature  $100\frac{2}{3}^{\circ}$ , and all the symptoms of chilliness and the fibrillary twitching had disappeared. On the fourth day after the animal had apparently recovered from the effects of the above dose, a similar amount of the same meat extract was given and the above-mentioned symptoms promptly returned, but were not quite so pronounced, and no nystagmus could be detected. On the third attempt to reproduce the preceding symptoms in this monkey, in spite of the fact that 15 grm. of the meat extract were given, the exophthalmos did not recur; the pulse and the temperature, however, increased slightly, and the appearance of chilliness, although prominent on the morning after the dose had been given, disappeared during the afternoon of that day.

Five weeks after the operation another 15 grm. dose of the meat extract was given to this monkey, which had apparently become perfectly normal again, and except for a temporary increase of twenty beats in the pulse-rate and a little transient diarrhoea, no effect followed. At this date the residual portion of the thyroid gland could be distinctly felt and was evidently much increased in bulk, and judging from the experiences of Wagner, Horsley, von Eiselsberg, Halsted and Breisacher, I conclude that a true hypertrophy of its glandular elements had probably occurred.\*

Thus, as had been previously anticipated, when sufficient functional activity of the residual thyroid had been regained, the increased susceptibility of the monkey to relatively small doses of the harmful substances that were contained in that particular sample of commercial meat extract became less and less, until finally with the restoration of normal metabolism the normal degree of susceptibility was reached.

Although partially thyroidless monkeys, and probably thyroidless dogs, and even men with disturbance of the activity of the thyroid gland, appear to be less capable of resisting the action of certain substances that are prone to arise in food-stuffs derived from the flesh of animals, further evidence is certainly desirable before it can be de-

\* This hypertrophied residuum of the left lobe was removed after a period of 20 months. Although the animal possessed a well differentiated aortic body, 2.5 mm. in diameter, and was given fresh thyroid, iodothyroine (Beyer), and thyrocolloid prepared according to Hutchison's method, by mouth and by injection, after it had become myxoedematous, it did not survive longer than five weeks.

nitely asserted that the intoxication which follows the ingestion of those substances is in reality entirely identical with that variety of intoxication of which the cachexia consists. It is true that the experiments of Bogoslowsky and of Kemmerich, as well as the other experiments which have been related in Part I of this paper, point to the fact that an intoxication, with some of its symptoms usually very similar to some of those manifested during the cachexia, can be produced in normal rabbits, and even in monkeys, by means of large doses of meat extracts prepared from meat that has been kept for a variable length of time; but as my experiments in this chapter show, the symptoms of the cachexia will develop in the totally thyroidless dog or monkey when only cereals compose the diet both before and after the performance of the thyroidectomy. It is obvious, therefore, that although certain toxic substances which a meat diet may or may not contain, may still further poison the thyroidless animal, other factors probably arising within the organism, as Ewald has pointed out, must evidently be considered as playing a far more important part in directly producing the cachexia. As regards the experiments in the partly thyroidless monkey, it is evident that the most justifiable deduction from the results is that, owing to the disturbed general metabolic condition that temporarily results from the partial loss of the thyroid glands, the natural resistance of the animal to a certain toxin-containing food was greatly lessened, and in consequence the susceptibility of the monkey to such poisons became for the time being more prominent.

3. *Birds.* In Part I I found that I was not able to produce any signs of an intoxication in a normal cock by the administration of very large doses of various decomposed animal tissues, consequently it was determined to perform as complete a thyroidectomy as possible and observe if any of the symptoms of an intoxication or of the cachexia could be produced in this bird by a meat diet, for it is stated that an apparently total bilateral extirpation of the thyroid and accessory thyroid bodies does not produce the symptoms of the cachexia in chickens (Allara \*) and pigeons (Ewald and Rockwell †).

\* Allara, *Lo Sperimentale*, 1885, p. 281.

† Ewald and Rockwell, *Pflüger's Archiv*, xlvi (1890), 160.

It is questionable, however, whether the preceding statement is to be accepted as final, for, as Horsley remarks, duration of life after the operation may play in thyroidless birds the important part it does in some other orders. Also the importance of bits of accessory and of embryonal thyroid tissue situated in unsuspected locations must not be forgotten, for, thanks to Gley, the importance of these apparently insignificant and easily overlooked bodies has been clearly demonstrated in rabbits and, thanks to Christiani, in rats.

After extirpating the thyroid and all of the adjacent accessory bodies that I could discover in a cock, it was fed for a number of weeks on meat, fresh, stale, and even slightly putrid, but no signs of an intoxication or of the cachexia could be detected. Wishing next to determine if the thyroidless bird had become susceptible to the substances contained in many of the dried thyroid preparations, it was given daily for three weeks large amounts (15 to 20 grm.) of the most toxic thyroid preparations that I had on hand. Lastly, I gave it by means of the tube the concentrated bouillon from 800 grammes of very stale sheep's thyroid, but none of these substances produced the least effect and the bird is still under observation.

Until, however, a careful dissection and a microscopical examination of possible residual accessory thyroid tissue is made. I refrain from drawing any conclusion from the above negative results.\*

PART III. SOME OF THE PHYSIOLOGICAL ACTIONS OF CERTAIN ANIMAL  
EXTRACTIVES.

A. *The thyroid extractives and their influence upon the acute cachexia.*

1. *Historical introduction.*

In Part I it was stated that although the results of my experiences fully warrant the conclusion that the ingestion of even large amounts of the finely minced, absolutely fresh, raw thyroid gland for a considerable period of time is not followed by any signs of an intoxication, or by any symptoms that resemble in the least those of exoph-

\* Since the above was written the bird has been killed, more than six months after the thyroidectomy. It must be admitted that the above experiment merely verifies the previous statement that normal cocks seem to be insusceptible to decomposed animal foods, for several small bits of tissue were found in the region of the right thyroid body that proved under the microscope to be accessory thyroid tissue. Probably these small bodies had hypertrophied from very minute embryonal accessory thyroid bodies, else I should not have failed to notice them at the time of the operation.

thalmic goitre, a certain amount of time might be necessary for the active principle or principles to develop in the freshly removed gland, exactly as is usually the case with an enzyme. In fact, a number of observers (White and Davies,\* Greenfield,† Notkine,‡ and others) hold that the active substance of the thyroid gland is probably an enzyme in character. In consequence of the preceding possibility it was found necessary to isolate the various components of the raw or of the dried complex thyroid material, and determine which of them is the active principle, and also if this active principle will induce an intoxication if given by the mouth in large doses to monkeys and other lower animals.

In conjunction with the experiments with the substances obtained from the thyroid gland, comparative experiments were also made with substances derived by similar methods from other animal glands and from muscle, and as some of the results that were obtained in the latter experiments have not been, as far as I know, hitherto described, a brief account of them is also included.

Evidently from the recent publications of Fraenkel, Baumann and Drechsel, a number of independent seekers after the active principle supposed to be contained in the thyroid gland have been at work at the same time as myself, and the preliminary results of one of these observers, Drechsel,§ agree in many respects with my own independent observations, although the chemical process advocated by Drechsel differs somewhat from one of those employed by me.

S. Fraenkel || states in a preliminary report, read before the Society of Physicians of Vienna, November 22, 1895, that he prepared cold or warm extracts of known quantities of dried sheep's thyroid, and after precipitating the albumins by acetic acid, he found the filtrate active, reaching this conclusion from the fact that after drinking the neutralized filtrate daily for six days he lost 300 grm. per day during that period. By the process very analogous to that frequently used for the extraction of creatin—a process also used by me in general for extracting the fresh

\* White and Davies, *Brit. Med. Journ.*, 1892, ii, 966.

† Greenfield, *ibid.*, 1893.

‡ Notkine, *Sem. méd.*, 1895, p. 138.

§ E. Drechsel, *Centralbl. f. Physiol.*, ix (1896), No. 24.

|| S. Fraenkel, *Med. Record*, Jan. 11, 1896.

raw thyroid and thymus glands, muscles, etc.—he obtained, after removing the lead by sulphureted hydrogen, a syrup which was dissolved in alcohol and precipitated by means of ether or of acetone. This precipitate crystallized after cleaning. The crystals\* were very hygroscopic, and permanent only in the “dried part.” They were soluble in water and in alcohol, but insoluble in ether. By decomposition of the precipitates produced either by phospho-tungstic acid or by phospho-molybdic acid, with barium hydrate, a free base was obtained in the form of a markedly hygroscopic syrup. The results of the elementary analysis of the acetic salt led to the empirical formula  $C_6H_{11}N_3O_5$ . To the pure substance he applied the term “thyreoantitoxin.” The physiological tests of this substance are also described by him, and will be referred to in detail later on by me.

Baumann † was the first to discover that iodine, probably in combination, exists in the thyroid gland, and concluded from Roos’‡ results in the reduction of goitres by the iodine compound that this compound, or “thyreo-iodine,” is probably the active principle of the thyroid gland. The method of isolating the thyreo-iodine is not described in Baumann’s article.§

Drechsel, after trying numerous undescribed methods, chose the following one which led to crystalline products:

The fresh pig’s thyroids were minced, and repeatedly extracted with water at 35° C. The aqueous extract after acidifying was boiled, filtered and evaporated to a syrup, which was filtered after standing. The filtrate was precipitated with phospho-tungstic acid, and this precipitate, after being washed free from chlorine, was repeatedly boiled in water until no more would dissolve, the insoluble part being finally dissolved in an ammoniacal solution. Both solutions were then decomposed with baryta water, the ammoniacal being previously treated with  $CO_2$  and then warmed until all ammonia had been driven off. The filtrates were then exactly neutralized with  $H_2SO_4$ . The residue of both solutions crystallized. According to Drechsel both of these crystalline products proved active when fed to thyroidectomized dogs, although not very markedly so. Fraenkel’s base this author considers as identical with one of the two he describes, although Drechsel does not specifically state

\* S. Fraenkel (*Wiener med. Blätter*, 1895, 13-15) considers these crystals as the acetic salt of the base thyreo-antitoxin.

† Baumann, *Zeitschr. f. physiol. Chem.*, xxi (1895), 319.

‡ Roos, *ibid.*, xxi (1895), 19.

§ Baumann and Roos, *ibid.*, xxi (1895), 481. See appendix for method, etc., p. 217.

that he has obtained the two crystalline substances in a state of absolute purity. The comparative action of these substances on the blood pressure is also not referred to in that preliminary communication.

As the above-mentioned observers point out, the only way that one can possibly determine which is the true active substance or substances of the thyroid is to try to isolate the various substances and try them, preferably first on the lower animals after removal of the thyroid gland, and later on myxedematous men. The former I have done, but owing to lack of human myxedematous material I have not had an opportunity to test their action in that disease. The various substances were fed in several experiments to totally thyroidless dogs, but as these animals very early in the cachexia refuse food of any description and later swallow fluids either with great difficulty or not at all, the results, even when the minced raw gland was given, were so discouraging that I concluded to inject the solutions of the various extractives from which the albumin and nucleo-proteids had been removed. Naturally, as a control to these experiments, equal or considerably larger amounts of the extractives were also injected into normal animals and the effects carefully noted; for, judging from the previous experiments of Chamberland and Roux,\* who noted toxic symptoms to follow subcutaneous injections of an alcoholic extract of spleen, and of Charrin and Ruffer † with bouillon, I considered that very probably all animal tissues would yield extractives which, if given subcutaneously in very large doses, would prove to be poisonous to many of the lower animals. From my investigations on this subject, so far as I have pursued them, I have no reason to alter that opinion.

## 2. *Methods of extraction.*

Of the various processes used by me in extracting the thyroid, thymus, various other glands and muscles, the following three appeared to yield the best results. One of these processes, namely, that frequently employed for the extraction of creatin, is almost identical with that described by S. Fraenkel, but the active mixture that I have gotten from the fresh raw thyroid by that method does not appear entirely to correspond to the alcohol-soluble crystalline substance that he describes. Furthermore, the physiological action upon the blood pressure appears to differ markedly from that obtained by him with the thyro-antitoxin. The third process, although yielding a considerable amount of a gummy, resinous extract, which apparently also

\* Chamberland and Roux, *Annales de l'Institut Pasteur*, 1888.

† Charrin and Ruffer, *Soc. de Biologie*, Jan. 26, 1889.

contains that thyroid principle or principles (Drechsel) which are beneficial to thyroidless dogs, is open to the objection that probably a certain percentage of the active material is lost from the employment of alcohol in the method, for, according to my experience, the more nearly absolute the alcohol the smaller is the amount of the thyroid substance that remains in solution. Briefly, the three methods most frequently employed by me for the extraction of the thyroid were as follows:

(a) The absolutely fresh raw thyroids (sheep, ox, calf) after mincing were mixed with about two volumes of cold distilled water and stirred briskly for twenty minutes. The mixture being continually stirred, was extracted for about twenty minutes at a temperature of 38° C. and then boiled for ten minutes. This hot bouillon was filtered through a fine linen cloth and the solid residue thoroughly expressed by means of a powerful press. After the grease had been skimmed off, the cool filtrate was acidified with acetic acid, boiled for ten minutes, filtered through fine paper and the cooled filtrate refiltered through paper. After evaporating this filtrate to a syrup and filtering again, the latter, after the acetic acid had been driven off by heat, was dissolved in a saturated solution of ammonium sulphate, and additional crystals of this substance were added to insure complete saturation of the whole mixture, which after standing was filtered through paper. The filtrate was decomposed with baryta water, CO<sub>2</sub> passed through the filtrate and all ammonium carbonate driven off by gentle heat. After repeated filtrations and evaporation a final brownish, waxy, hygroscopic residue was yielded. As the result of comparative tests of the various precipitates and filtrates, it was concluded that this residual waxy mass contained some of that part of the thyroid material which considerably influenced both the severity of the symptoms and the course of the cachexia in totally thyroidless dogs.

(b) The well boiled acidified thyroid bouillon, prepared as in the preceding method, was precipitated by lead acetate, filtered and excess of lead removed by means of sulphureted hydrogen. After testing the filtrate for lead it was evaporated on a water bath and, when it had reached the consistency of a thick syrup, it was carefully refiltered while hot. The syrup was further evaporated, ultimately yielding a dark brown wax-like mass, similar in appearance to that which had resulted by method *a*.

(c) To the filtered acidified thyroid bouillon, after it had been evaporated at 100° C. to about one-fourth of its original volume and allowed to become cold, one-half volume of ether was added and the whole well shaken in a stoppered vessel for about twenty minutes. After standing,

the supernatant ether was decanted and the aqueous remainder filtered, all ether being finally driven from the filtrate by means of gentle heat. Three volumes of 95 per cent. alcohol were then added to the filtrate and the whole allowed to stand for twenty-four hours, the ensuing precipitate being then carefully filtered off. The filtrate was finally evaporated at 100° C., being repeatedly filtered during this process until the whole had become as dry as possible. This residue was dissolved in distilled water by means of heat, and after filtering the filtrate was evaporated at 100° C. until the reaction of the resulting brown gummy wax had become neutral or faintly alkaline. This wax appeared to contain most of the active substance, although the alcoholic precipitate appeared to be slightly active also, due to the fact, as I found out by other methods, that the waxy mass is but extremely slightly soluble in strong alcohol.

Although it was evident that these waxy hygroscopic masses which appeared to be very nearly similar, though obtained by three different methods, assuredly contained some of the thyroid principle—for subcutaneous injections of a 2 per cent. aqueous solution of any of the three considerably prolonged the existence of totally thyroidless young dogs—it was probable that the waxy mass also contained a number of other extractives. Consequently a number of methods were employed to subdivide the waxy masses and obtain the desired substance in a purer form, possibly in a crystalline condition. An aqueous solution of this wax was then precipitated, either by phospho-molybdic acid or by phospho-tungstic acid, and after carefully washing the precipitates with a 10 per cent. solution of the respective acids they were boiled in distilled water, the solutions allowed to become cold, filtered, and the filtrates decomposed with baryta water, neutralized with sulphuric acid and evaporated to a very thick syrup, which, on being set aside, deposited crystals of various kinds (5 to 8 varieties). The portion of the precipitate that was insoluble in boiling distilled water was dissolved as in Drechsel's method, in water rendered slightly alkaline with ammonia; the ammonia removed by CO<sub>2</sub> and heating the filtrate until all ammonia had been driven off. This residual solution was finally decomposed with baryta, neutralized with H<sub>2</sub>SO<sub>4</sub>, evaporated on a water bath and set aside to crystallize. But few crystals appeared when phospho-molybdic acid was used, the chief residual yield appearing to be an oily base-like substance. When phospho-tungstic acid was used the proportion of the various crystals was larger. These ultimate mixtures, containing 5 to 8 varieties of crystals in their oily mother liquor, were injected into totally thyroidless dogs, and each found to be active and capable of prolonging the lives of totally thyroidless dogs equally as well as injections of the mixed waxy extrac-

tives, and even better, according to my experience, than injections of unboiled aqueous or glycerine extracts of fresh raw thyroid glands.

Various attempts were made to separate the oily mother liquid from the various crystals and obtain the various crystals and the mother liquor in a state of absolute purity, but these efforts have been up to the present unsuccessful, consequently I cannot positively assert whether it is the oily or the crystalline substances that are active. From two of these ultimate extracts, one by the phospho-molybdic acid method, and the other by the phospho-tungstic acid method, I succeeded by using amyl alcohol in removing some of the mother liquid, leaving most of the crystals undissolved, but neither the activity of the oily mother liquor nor that of the remaining crystals appeared to have been diminished, as well as could be judged from a test upon two totally thyroidless dogs. Thus it cannot be inferred that only the oily substances are probably the active parts of these two ultimate extracts. Whether one of these oily substances is the same as the base isolated by Fraenkel I cannot say, for, though ether precipitates it from an aqueous alcoholic solution, anhydrous alcohol dissolves scarcely a trace of it, and none at all after this trace has been several times precipitated by anhydrous ether and the supernatant alcohol and ether decanted. Fraenkel's active base appears from his account to be so soluble in alcohol that the alcohol-ether method is employed in extracting it in a pure form from the crude syrupy mixture. Further, both of the substances that I get give rise to very marked, though transient, alteration of the blood pressure when they are injected, singly or combined, directly into a vein, but Fraenkel states that no fall of the blood pressure follows the intravascular injection of his base, although acceleration of the pulse occurs.

The two active though impure products that I get from the wax extracted from the fresh thyroid probably correspond to those described by Drechsel, although it must be admitted that I have not been able to assure myself that the activity of the two products depends upon the presence of two different chemical bodies, for, as has been intimated above, I have isolated neither principle in a chemically pure state. It is possible that the two active products that are yielded by the waxy mass which contains various thyroid extractives owe their activities to an imperfectly performed chemical separation at the point when the phospho-tungstic or phospho-molybdic precipitates were partially dissolved in boiling water. I inferred this possibility from the fact that after dissolving one of the crystallized products in dilute alcohol and precipitating with ether, recrystallizing and removing as much of the mother liquor as possible with amyl alcohol and recrystallizing the undissolved crystals,

the majority of the latter changed in form and crystallized exactly in the stellate form assumed by the majority of crystals yielded by the other crystallized thyroid product. An ultimate analysis of these crystals isolated in a pure form would have settled the question, but this, however, has not been attempted with my present chemical appliances.

It is noticeable that Drechsel does not specifically state in his preliminary communication that he has isolated the two principles in an absolutely pure form, nor does he state which of the crystalline substances occurring in the active residues are the true active principles. Drechsel's method, however, is evidently a more direct and simpler way of obtaining these substances than any of those mentioned above, and I have lately endeavored to isolate by that method the two active thyroid bases of Drechsel in pure form. Up to the present my several attempts have met with no better success in the case of these partially crystalline residues, which the method appears to yield, than they previously did when I attempted in the summer of 1895 to do the same thing with the residues obtained by one or other of the three methods which I have described above. The investigations on this subject, nevertheless, are still in progress.

#### *B. The effects of the thyroid extractives on thyroidless dogs.*

In the preceding section it was stated that the daily subcutaneous injection of even comparatively small doses of a 2 per cent. solution of the gummy, waxy mixture of the thyroid extractives before being treated with phospho-molybdic or phospho-tungstic acid, etc., into totally thyroidless dogs appears to exert a most decided effect upon both the course of the cachexia and upon the severity of its symptoms, consequently it was concluded that the active palliative thyroid substance was present in these extractives. I do not intend to convey the idea that I succeeded in keeping the totally thyroidless young dogs alive by this means for any great period of time, for I have never been able to do this either by the injection of fresh aqueous thyroid extracts or by feeding large amounts of fresh thyroid glands to the dogs, because all of my totally thyroidless dogs died in spite of the injection of the extractives in from ten to eighteen days after the operation. When the fresh aqueous unboiled thyroid extracts were injected eight to twelve days proved to be the greatest length of time that the lives of the thyroidectomized young dogs could be prolonged, provided the dogs

had no tiny aortic or other accessory thyroid bodies. When compared with the results by the feeding methods which have been described in Part II, these results are very striking, for not only was the duration of life after the thyroidectomy further increased, but the intensity of many of the symptoms of the cachexia was very much lessened.

Briefly, the effects upon the cachexia of the subcutaneous injection of the fresh *crude* aqueous thyroid extracts were somewhat analogous to those of Gley and were as follows:

If the injections of the thyroid extract were begun on the day of the operation in daily doses of from 2 to 10 ccm. (each ccm. = about 1 grm. of raw gland), the dose varying with the weight of the dog at the outset, all of the dogs at times had mild convulsions, constant fibrillary muscular twitching and unsteadiness of gait, while occasional attacks of tetany were also prominent. When signs of an approaching dyspnæic attack were observed the injection was repeated. As a result the dyspnœa generally disappeared in the course of forty-five minutes or one hour. From the third or the fourth day the emaciation appeared to become more rapidly progressive, and steadily continued until the death of the animal. By the fifth or sixth day all food was refused and milk had to be given by the stomach tube. Even the milk was usually vomited in spite of previous cocaineization of the fauces. Finally death occurred about the tenth or twelfth day, unless the dogs happened to have a sufficiency of small aortic or other accessory bodies. In the latter case the symptoms progressed more slowly, the tremor and restlessness frequently disappearing for several days at a time. In one dog with a large accessory aortic body all the symptoms disappeared after the lapse of ten days. When no injections of the fresh aqueous thyroid extract were given to the totally thyroidless dogs until convulsions had appeared, the only noticeable effect was a temporary moderation of the fits, the tetany and dyspnæic respiration being also considerably lessened, but the betterment was merely transient. In spite of frequent repetitions of the injections the fits recurred after one or two hours and continued to occur at intervals until the animals died, usually five or six hours after the attack had begun.

When enough of a sterilized 4 or 6 per cent. aqueous neutral solution of the extracted waxy substance was daily injected into the thyroidless dogs before any fits or dyspnœa had developed, the effect upon the symptoms of the cachexia was clearly more pronounced than that from the crude aqueous extract of the raw gland, and the prolongation of life was

greater, the dogs not dying until the fourteenth or eighteenth day. In these dogs also the pronounced emaciation steadily progressed from the second day after the thyroideectomy until the end, although artificial feeding with milk was tried.

When about 30 minims of the 4 per cent. solution was tried as a beginning daily dose for a dog weighing 5500 grm. the quantity was evidently too small, for on the morning of the fourth day the dog was found in a comatose condition, breathing stertorously and having numerous fits. 100 minims were injected and one hour afterward the dog was able to stand up, although considerable stiffness remained in the hind legs. Two hours after, as the respiration of the animal had become very rapid and panting, and the cramps more pronounced, 75 minims were injected. On the next morning only moderate fibrillary muscular twitching and a slight degree of restlessness were present, the conjunctivitis, coryza and tetany having completely disappeared. From this date until the day of the animal's death 100 to 150 minims were injected daily, but as the dog would eat nothing and could not be induced to retain food artificially introduced into the stomach, death finally occurred on the fourteenth day.

When the fits were allowed to begin before any injections of the extractions were given I succeeded in causing the symptoms to subside only in those dogs that were injected very shortly after the beginning of the fits. If the dogs had been allowed to remain in the rigid, comatose condition and have numerous fits, although very large doses appeared to slightly lessen their intensity and to diminish the rapidity of the respiration, the totally thyroidless dogs did not recover from the attack, but died in from four to eight hours after it had begun.

After separating the gummy mixture of extractives into the two partially crystallized residual portions by treating it with phospho-tungstic acid, phospho-molybdic acid, etc., and injecting the residual portions separately or in combination into various totally thyroidless dogs, the resultant average prolongation of the animals' lives was practically about the same as if an equivalent quantity of the 4 per cent. watery solution of the unseparated waxy mixture of extractives had been used. When comparatively large quantities of either one of these two partially crystallized residual substances were injected, each of which appeared to be composed of crystalline products mixed with more or less oily mother liquor, the palliative effects upon the cachexia apparently differed in no way from those produced by injections of both combined in smaller amounts. All of these dogs died about the fourteenth or sixteenth day in spite of the palliative substance that had been introduced under the skin.

As the results of a number of further experiments with the thyroid extractives derived either by Fraenkel's or by Drechsel's methods have been invariably about the same as the above—not even as good when the fresh uncooked watery extract of the raw gland has been given—I am firmly convinced that it is impossible to prolong for any great length of time the life of a young dog that has been deprived of absolutely all thyroid and accessory thyroid tissue, by feeding the raw gland to the dog, or by injecting the water-glycerine extracts of the fresh raw gland or the concentrated, highly purified extractives isolated from the raw gland—an extended conclusion which has apparently been already indicated by the well-known experiments of Vassale, Gley, Hofmeister, Hertzen, Schwartz, Godart, Edmunds and others, with the crude aqueous or glycerine thyroid extracts.

### *C. The toxicity of the thyroid extractives.*

It is evident, as I have remarked, that some of the active palliative thyroid substance is assuredly contained in the gummy extracted mass, and as absolutely fresh raw thyroid gland has been used for the preparation of this waxy mass, it is also evident that this active substance does not exist in the fresh raw gland, for an interval of only about twenty minutes occurred between the slaughtering of the animals from which the material was taken and the beginning of the extraction. Furthermore, from the manner of its extraction it is certainly not an enzyme, for boiling does not appear to affect its activity in the least.

Having obtained the active substance in this more purified concentrated form, it was necessary to give large doses of it to susceptible animals before I could pronounce upon its toxicity. Finally, from the indications of my previous results with the dried or the stale thyroid preparations, it was desirable to make comparative tests with extractives derived from the preparations which had proved to be very toxic to rabbits and monkeys when administered by the mouth.

Briefly summed up, I find that after giving the mixed extractives from the fresh gland to rabbits by the mouth in doses of 4 grm. (= about 220 grm. fresh thyroid) per kilo of rabbit, and continuing the same for even five or six days, the rabbits do not appear to manifest any symptoms at all, nor does the temperature rise above the normal.

To monkeys also were given as much as 4 grm. per kilo of body weight, but with entirely negative results. With the extractives from an average dried commercial thyroid preparation the results were totally different and deserve to be more fully described.

The extraction of the commercial dried thyroid gland was made by the acetate of lead method, the same method being also used for the fresh raw control material, and 0.25 grm. of the residual gummy mass, which the dried thyroid also yields, was given to a rabbit by the mouth. At the end of fifteen minutes the respirations of the rabbit had become about 225 per minute and were very shallow; the heart-beat was so feeble and rapid that it could not be counted. Considerable weakness in the hind legs was also present. In twenty minutes it lay sprawled out on its belly, with marked exophthalmos, and occasionally a slight muscular twitch would occur in one or other of the legs. Pronounced horizontal nystagmus next developed and continued for about ten minutes. Towards the end the respiration became slower and shallower and finally ceased, about forty-five minutes after the introduction of the dose. It was evident, therefore, that the toxic properties of the dried material had not been wholly, or apparently even partially removed by the chemical processes employed to obtain the gummy mass of extractives, which in this case were contaminated with a considerable number of crystals of chloride of sodium. Having treated the gummy mass, liquefied by heat, several times with 90 per cent. alcohol, I found that the undissolved portion still preserved its activity as far as its palliative influence upon the cachexia was concerned; also that this active residue had apparently been deprived of those constituents which were toxic to monkeys and to rabbits. Thus large doses of this active residue could be given to these animals by the mouth without producing any of the symptoms that the dried powdered gland had produced.

With the portion dissolved by washing the mass several times with the 90 per cent. alcohol, precipitating with anhydrous ether and dissolving the cleaned precipitate in water, the resultant toxic effects were extremely marked. As an average illustration of the effects of small doses to rabbits, the photograph reproduced in Plate XIV is appended. This rabbit was given 0.1 grm. by the mouth and the photograph was taken about twenty-five minutes after this dose had been ingested. The characteristic exophthalmos is fairly well seen, as well as the sprawled out position due to paresis of all four legs. Shortly after this picture was taken a marked, though temporary, horizontal nystagmus developed. The number of heart beats increased from 192 to about 286 per minute:

the temperature from  $103\frac{1}{2}$ ° F. to  $105\frac{1}{2}$ ° F., and the respirations from 60 to 80. At the end of four hours the rabbit was beginning to recover and by the next morning the above symptoms had to all appearances entirely disappeared. The rabbit now weighed 105 grm. less than it did on the previous day, but this loss was regained in the course of several days. When larger doses (1 grm.) were given to rabbits by the mouth the above symptoms rapidly developed, but were of very short duration. All the animals died within from 30 to 45 minutes, and two of the four so treated exhibited shortly before death an occasional spasmodytic twitch in one or other of the extremities.

As this toxic and evidently impure ether-precipitate had been extracted from the gummy mass by means of dilute alcohol, I naturally supposed that at least a small part of the apparently harmless but active thyroid principle had also been dissolved by the dilute alcohol, and this undoubtedly was the case, although the palliative effect of this mixture was much less pronounced than its toxic effect. By washing the precipitate, produced by adding ether to the alcoholic solution, several times with boiling absolute alcohol, decanting the supernatant alcohol and injecting an aqueous solution of the oily remainder, which was insoluble in the absolute alcohol, into a totally thyroidless dog, the course of the cachexia appeared to be modified equally as if the mixed active extractives from the fresh raw gland had been used. The decanted alcoholic portion, when evaporated and dissolved in water, did not seem to possess this power, for the thyroidless dogs that were injected with small quantities of the solution promptly died between 50 and 60 hours after the thyroidectomy. If large doses of this solution of toxic substances were injected into thyroidless dogs when they exhibited a considerable degree of tetany and pronounced prodromal signs of an attack of dyspnea, the effect appeared to be that the symptoms of the cachexia became entirely altered and gradually gave place to those produced by the toxic substances. As some of the latter symptoms were to a certain extent the diametrical opposites of the former, the condition of tetany rapidly disappeared and all four legs soon became weaker, more flaccid, and finally completely paralyzed, so that the dogs lay sprawled out upon their bellies. Slight exophthalmos replaced the enophthalmos and, except for an occasional muscular twitch in the legs, no general convulsions, as far as I know, occurred. But as all these dogs were found dead on the morning following the afternoon on which the injections had been made, convulsions may have occurred during the night. Although it cannot be said that the lives of these dogs were prolonged by the toxin-containing mixture which the alcohol extracted from the waxy mass of extractives

made from the dried thyroid material, the temporary and usually rapid effects following its injection in doses capable of producing the intoxication with its accompanying paralysis, etc., might, it is conceivable, lead one to believe that a large dose of the proper palliative thyroid substance had been injected, especially if the dogs were observed only during the transitional period between the subsidence of the tetany and the onset of the paralysis. Such a conclusion would certainly be erroneous, for, though the thyroidless dogs possibly got in this instance a very small amount of the proper palliative thyroid substance which the strong alcohol had dissolved, I have been able to produce identically the same effects by injecting thyroidless dogs with solutions of poisonous extractives obtained from various commercial meat extracts which also produced the above-described variety of intoxication in normal rabbits.

In the waxy mass of thyroid extractives prepared from the dried glands there appear to be at least two substances with entirely different physiological actions. These are:

(a) The principle or principles (Drechsel) that act beneficially upon the experimental acute cachexia thyreopriva.

(b) The principle or principles that are capable of producing an intoxication in certain animals when given by the mouth or by subcutaneous injection.

The first (a) is evidently the same as that which can be extracted from the fresh raw gland, and is apparently innocuous when given by the mouth to rabbits and to monkeys in extremely large doses. The second, or the toxic principles, does not prolong the lives of the totally thyroidless animals and, moreover, does not appear among the extractives when the perfectly fresh thyroid gland is employed. From their chemical behavior it cannot be said that they are enzymes. Nor can it be said that the intoxications that they produce are specific for the thyroid only, for substances capable of producing intoxications similar in every respect to those produced by the thyroid toxins appear to exist in the extractives from other stale animal tissues, muscles, spleen, thymus, etc. It is evident, therefore, that the thyroid substances which prove toxic by way of the stomach to normal rabbits, monkeys and probably also to men, arise during the post-mortem chemical alterations which the constituents of various animal tissues, and especially those of the thyroid gland, are more or less prone to undergo.

Are the mixed extractives of the fresh thyroid gland poisonous to animals when large doses are injected beneath the skin? Most certainly, according to my experience, but not more so, or even frequently not as much so, as some of the extractives derived by similar chemical methods

from the fresh suprarenal capsules, the spleen, the liver, the muscles, the salivary or the lymphatic glands, the spinal cord and the blood (dog). But because all the above dead animal tissues will yield extractives that are poisonous when injected in rather large doses under the skin of an animal, one is not, however, justified in concluding forthwith that these poisonous extractives normally enter the blood in this form from the living tissues of the body. Consequently I do not think that the subcutaneous injection of the mixed extractives obtained from a supposedly internal secreting ductless gland by the above chemical methods, which very probably bring about extensive molecular changes in the original living constituents of the glandular tissue, truly indicates whether the supposed normal internal secretion, as it exists in the living body, can be toxic or not if it is produced in too great a quantity.

The only reliable method of testing whether or no the impure active thyroid principles are capable of producing an intoxication is to give large doses by the stomach, for both chemical and experimental evidence clearly demonstrate that their palliative activity on the cachexia is not lessened when they are absorbed from the alimentary canal.

Clinical and experimental tests have also clearly shown that when certain thyroid preparations are taken into the stomach by healthy or by diseased individuals they are capable of producing an intoxication with symptoms resembling in some respects those of Graves' disease. The digestive juices, therefore, destroy neither the active palliative thyroid principles nor the toxic principles. The raw fresh gland does not appear to contain the latter, but does contain the former, an apparently harmless substance even when ingested in enormous amounts; therefore, if the negative results of the tests by the stomach be considered valid, it is extremely probable that in exophthalmic goitre it is not the supposed increase of the normal active palliative thyroid principle which is responsible for the symptoms of that disease.

Are the toxins which one only finds in thyroid material that has been kept for a number of hours after the death of the animal, in reality identical with those toxic substances which the adherents of the hyperthyroidism theory of exophthalmic goitre claim the enlarged, or even the unenlarged thyroid gland is contributing to the blood? Judging from the extremely small amounts, frequently but 15 grains, of many of the dried thyroid preparations that will produce death in a strong, healthy monkey, I should say that the substances are not the same. The toxicity appears proportionately too great, for should a considerable quantity of such substances continuously enter the blood, I judge from my results in monkeys that rapid death would occur.

When but fifteen grains of a normal sheep's thyroid gland contain enough of these toxins to kill a monkey or a rabbit, it is legitimate to think that the enormously increased secretion of the supposedly over-active and often extremely enlarged thyroid in exophthalmic goitre would contain many times the quantity of toxins that are contained in fifteen grains of a normal gland. Did the internal secretion of the living goitre contain proportionate amounts of toxins identical with those of the dried normal gland, either a considerable degree of tolerance of rather large amounts of the toxins would probably follow, as the active secreting glandular elements of the thyroid gradually increased in amount, or if such tolerance were not established, very rapid death would occur, just as it does in an animal poisoned with the dried juice, or as it frequently does in man just after the partial excision of the struma. The result in the latter instance is due, according to the majority of surgeons (Kocher and others), to the inundation of the system by the thyroid juice unavoidably squeezed into the lymphatics during the manipulations of the operation, or to absorption of some of the poisonous juice which, according to Jaboulay, flows in enormous quantities from the gland after the performance of exothyropexie. When the enlarged living gland is continually adding such enormous amounts of a juice which ought, therefore, to contain an enormous amount of extremely toxic products, the degree of toxicity being judged by that which can be present in such small quantities of the dried normal sheep's thyroid, it is extremely probable that a certain degree of tolerance would occur, else the average case of exophthalmic goitre would not be liable to progress in the usual chronic manner. If the system of a patient with exophthalmic goitre become partially accustomed to the large continued inflow of the toxin-containing thyroid secretion, and were the poisons of that secretion identical with those that one usually finds in the poisonous thyroid preparations, one would hardly expect that the ingestion of the additional quantities of the supposedly similar toxins that are contained in one five-grain thyroid tablet per day for only one day would be followed by any very rapid and pronounced aggravation of the symptoms of the disease, especially as a considerable degree of tolerance of the thyroid toxins can be produced in some of the lower animals, and the same thing has been observed by clinicians to occur in man. Thus from a series of experiments on rabbits and monkeys I found that by very gradually increasing the daily dose of a poisonous thyroid preparation until relatively large amounts of the preparation could be ingested without producing any very pronounced symptoms, a fair degree of tolerance of the toxins that were contained in that variety of thyroid preparation

could be produced. For example, a monkey that had been accustomed in this manner to 3 grm. doses of a preparation, took that quantity daily for five days and showed but very slight symptoms, whereas, when another monkey of the same species, that had previously received no thyroid, was given 20 grains of the same preparation, the monkey died in five hours.

Even a slight degree of tolerance of the poisonous thyroid preparations does not, however, appear to be present in exophthalmic goitre, a fact fully substantiated by the reports of Auld, Dreyfus-Brisac, Béclère, Ewald, Ingalls, Nasse, Stabel, Starr, and numerous others besides myself. According to my experience some of the symptoms of the several cases of exophthalmic goitre, to which I very cautiously prescribed small doses of commercial thyroid tablets that were subsequently found by experiment to contain a considerable proportion of toxic products, became apparently aggravated as early as the morning of the second day of the treatment, although but two 5-grain tablets had been taken in some instances. But the finely minced, absolutely fresh raw sheep's thyroid did not produce, as I have previously pointed out, this exacerbation of the symptoms; in fact, the patients with Graves' disease seemed to bear both the fresh gland and the extractives of the fresh gland very well.

Further, although the urine of individuals with Graves' disease has been demonstrated by Chevalier, Boinet and Silbert, Albu and others, to contain a larger percentage of toxins than that of normal individuals, none of those writers has demonstrated that the toxins excreted in the poisonous urine of those patients were identical chemically with those met with in the poisonous thyroid preparations.

I may furthermore add that the various poisonous thyroid preparations do not invariably produce symptoms that resemble those of exophthalmic goitre. Some of the symptoms may resemble those of the latter disease, but frequently marked differences are met with in the experimental intoxication, and furthermore I reiterate that toxic substances that produce identically the same symptoms as those resulting from the ingestion of the poisonous thyroid material may develop in other animal tissues. The latter poisons vary also in quantity and quality just as the thyroid poisons do.

From the above evidence it cannot be said that it is by any means conclusively indicated that the toxic substances which may or may not be found in certain thyroid preparations are identical with those that many observers suppose are being contributed to the blood by the enlarged thyroid in Graves' disease.

## APPENDIX.

As practically the whole of this paper was already written before the publication of the second contribution of Baumann and Roos,\* in which is described the method of preparing the principle "thyro-iodine," which they consider to be the active substance of the thyroid gland, my experience with that body must be related briefly in this appendix.

Baumann's method is as follows: The thyroid glands are boiled in a 10 per cent. solution of sulphuric acid until a nearly complete solution of the glands occurs. The active substance is for the most part contained in the fine, flocculent precipitate which also contains fat, fatty acids and other substances. The precipitate is repeatedly boiled in 90 per cent. alcohol with the result that the "thyro-iodine" gradually goes into solution in the alcohol. The alcoholic solution is evaporated and the residuum triturated with milk-sugar. After extracting the triturate with ether and benzin, it is dissolved in a cold dilute solution of caustic soda. On acidifying this solution the thyro-iodine is precipitated. Having redissolved it in caustic soda solution and reprecipitated it with  $\text{HCl}$  or with  $\text{H}_2\text{SO}_4$ , the coloring matters are separated and the thyro-iodine is obtained, after drying, in the form of a brownish powder that is insoluble in water but soluble in caustic alkalies. By this process 25-30 per cent. of the original iodine combination is lost. The greater portion of the thyro-iodine is united in the thyroid gland to two proteid bodies, "thyro-iodo-globulin" and "thyro-iodo-albumin," and only a small portion of the thyro-iodine is present as such in the thyroid gland.

Roos † has lately investigated the toxicity of thyro-iodine—a preparation that had been manufactured by F. Bayer & Co. according to Baumann's process—and in reference to Lanz's conclusions that the symptoms of the thyroid intoxication are due partly to the action of the thyroid material, and partly to the action of decomposition products, writes, "Eine Ansicht, die durch die Versuche mit dem Thyroiodin, wobei von irgend welchen Verunreinigungen durch Bakterien oder Zersetzungsprodukte keine Rede sein kann, ihre Bestätigung erhalten hat." Incidentally, I must add in this connection that, judging merely from the odor of some of the bottles of thyro-iodine or rather "Iodo-thyrine" that were procured from that firm, one would be inclined to believe that a certain amount of decomposition had occurred in the original raw thyroid material, probably during the time consumed in collecting a large number of glands at the slaughter-house and forwarding them to the manufactory,

\* Baumann and Roos, *Zeitschr. f. physiolog. Chem.*, xxi (1896), 481.

† Roos, *Zeitschr. f. physiol. Chem.*, xxii (1896), 18.

where further time is consumed in carefully selecting the healthy glands. Be this as it may, however, Roos obtained (Exp. 2) from three doses (altogether equal to 18 grm. of raw gland) of this thyro-iodine preparation, headache, tremor, diarrhoea, palpitation and accelerated heart's action (108) with a temperature of 36.8° C. From larger doses (the equivalent of 18 grm. of raw gland given in two days) practically the same symptoms occurred, but the induced condition was never serious and the symptoms ceased after discontinuing the preparation. The experiences of Grawitz\* and of Hennig † with this commercial thyro-iodine preparation also confirm those of Roos.

On the other hand Ewald,‡ discussing the effects of thyro-iodine on man, remarks as follows: "Vorübergehende Anwendung des Thyro-jodins selbst bis zu 4 grm. pro die, hat keinen erheblichen Einfluss auf den Puls. Von Jodintoxication kann nicht die Rede sein, weil die Menge von Jod in der Drüse und ihren Präparaten sehr gering ist. Dagegen, werden Intoxicationsen überhaupt beschrieben, wie Steigerung der Respirationsfrequenz, Kopf- und Gliederschmerzen, Speichelbluss, Urticaria, Herzklopfen, Zittern, u. s. w. Oft genug tritt aber trotz Anwendung grosser Quantitäten sicher wirksamer Präparate keine Reaction ein." According to Schuster § even the ten- or twenty-fold dose of thyro-iodine causes no disturbance of the pulse in man, nor does the intravenous injection of thyro-iodine cause disturbance of the blood pressure or of the pulse (animals?). Nevertheless, Baumann || states, "Schilddrüsenpräparate mit welchen man jene Vergiftungserscheinungen überhaupt nicht hervorrufen kann, werden überhaupt wenig wirksam sein." Further, Baumann calls attention to the more rapid action of thyro-iodine as compared to that of the raw gland or thyroid tablets, and explains the occurrence on the ground that the thyro-iodine must first be separated from the constituents of the thyroid material by the process of digestion—a statement which, in conjunction with the fact that thyro-iodine is insoluble in water and exists in very small amount as such in the raw gland, is, to say the least, very irreconcilable with the previous observations of others as regards the activity of crude aqueous extracts in myxœdema.

As the preceding evidence concerning the toxic nature of thyro-iodine

\* Grawitz, *Münch. med. Wochenschr.*, 1896, No. 14.

† Hennig, *ibid.*, 1896, No. 14.

‡ Ewald, XIV. Congress f. inn. Med., Wiesbaden, April 11, 1896. *Wiener med. Wochenschr.*, 1896, No. 10, 376.

§ Schuster, *Wiener med. Wochenschr.*, 1896, No. 10, 379.

|| Baumann, *Münchener med. Wochenschr.*, 1896, No. 14.

derived from supposedly fresh thyroid material still leaves the subject in doubt, especially as all those observers who note the occurrence of toxic symptoms from the administration of thyro-iodine appear to have employed commercial thyro-iodine preparations which were obtained from the same firm, my own results with evidently active thyro-iodine preparations, prepared by myself and carefully tested for iodine, are appended.

After preparing a large amount of thyro-iodine according to Baumann's method and testing it for the presence of iodine, 2 grm. of the dried powder (unmixed with milk-sugar) were suspended in warm water, the acid solution was neutralized and given to a rabbit by means of the stomach tube. No immediate or remote effects on the heart-beat, the temperature or the eyes could be detected. No diminution in weight resulted.

A Java monkey was given 8 grm. of this preparation and two days later 10 grm. were given. As with the rabbit the result was negative.

To a patient with an enlarged thyroid gland daily doses of 1 grain of this thyro-iodine triturated with a little milk-sugar were given for a week. At the beginning of the treatment the circumference of the neck was  $13\frac{1}{2}$  inches; on the ninth day the neck measured  $12\frac{1}{4}$  inches. No disagreeable symptoms appeared and, as this particular preparation was exhausted, the patient was put on 1 grm. doses of the commercial "iodo-thyrene" (1 grm. = 1 grm. of gland or about 3 milligrammes of purified thyro-iodine). After a week's administration of the latter preparation the neck measured but 12 inches. So far no disagreeable symptoms have occurred in this patient from doses of the above amount, nor has the patient diminished in weight. Possibly were larger doses to be given to this patient this preparation might give rise to toxic symptoms similar to those that Roos has observed with preparations from the same source.

Owing to lack of opportunity the thyro-iodine preparation made by myself was not administered to a patient with myxoedema. I am, therefore, unable to pronounce upon its activity in palliating that disease, but, judging from the diminution of the goitre which occurred during its administration, I am of the opinion that the preparation could be properly considered an active one. The toxic properties of commercial thyro-iodine have not been investigated by me in animals, but the experiments of Ewald indicate that the toxicity of the various lots probably varies like that of the various commercial thyroid powders and compressed tablets.

According to Baumann \* the thyro-iodine is the only active thyroid

\* Baumann, *Münch. med. Wochenschr.*, 1896, No. 20.

principle, and from experiments on two dogs, he and Goldmann conclude that small doses of thyro-iodine are fully capable of completely replacing the thyroid function in animals (dogs) that have been deprived of that function through removal of the thyroid gland. Gottlieb's \* results with thyro-iodine in thyroidless dogs do not, however, agree with those of Baumann and Goldmann, and my own results in *totally* thyroidless dogs with concentrated extracts obtained from thyroid glands by means of salt solution, which, according to Baumann and Roos, extracts all of the iodine combination from the minced gland, lead to the belief that the conclusion of Gottlieb that thyro-iodine is not capable of keeping the thyroidless dogs indefinitely alive is perfectly correct. Moreover, other experiments with purified thyro-iodine yield results that fully confirm the above conclusion of Gottlieb.

From the clinical results of Ewald and others with commercial thyro-iodine in the treatment of myxoedema, it is evident that the thyro-iodine prepared by the method of Baumann does contain something that influences the symptoms of that disease. Wishing to find out whether or no the iodine-containing product which Baumann's method yields would furnish further residual products if it was treated according to Drechsel's method of extracting the thyroid gland, four grammes of the powdered iodine-containing thyroid product were boiled in distilled water, the decoction being neutralized by a small quantity of caustic soda solution. After this solution had cooled it was filtered and the filtrate precipitated with phospho-tungstic acid. This precipitate was treated as Drechsel describes and ultimately yielded a small quantity of gummy residue that contained no iodine, but which after being dissolved in water appeared to possess physiological properties similar to those of the residue resulting from the ammoniacal solution of the phospho-tungstic thyroid precipitate in Drechsel's process. The chemical nature and composition of the gummy residue obtained from the iodine-containing product that was extracted from the thyroid gland according to Baumann's method have not been investigated by me except to test it for iodine, therefore I am unable to give further information as to its origin.

It is evident that the above-described facts concerning the production of toxic symptoms similar to those of exophthalmic goitre still harmonize with the general results discussed elsewhere in this paper. Also it is readily conceivable that should the least post-mortem decomposition have occurred in any of the thyroid glands before being put through the various stages of Baumann's process, some portion of the toxic sub-

\* Gottlieb, *Deutsch. med. Wochenschr.*, 1896, No. 15, 235.

stance or substances might still contaminate the thyro-iodine, so that in the hands of some observers one lot of thyro-iodine would give rise to certain disagreeable symptoms, while other observers using thyro-iodine prepared from a different lot of thyroid material would not observe the occurrence of those symptoms, even though very large doses were given. Even in a city of the size of New York, where large numbers of sheep and oxen are slaughtered daily, it is exceedingly difficult to gather together large amounts of absolutely fresh thyroids from animals that have been killed but a few minutes, and it is extremely likely that when very large quantities have to be collected for the preparation of thyroid products on a commercial scale, and twelve to twenty-four hours elapse between the death of the animals and the extraction of the glands, some decomposition has occurred in the material.\*

#### D. *The effect of the thymus extractives upon the acute cachexia in thyroidless dogs.*

Concomitantly with the previously mentioned experiments with extractives derived from the thyroid gland, I have also carried on a series in which the thymus gland of the young ox served as the source of the extractives.

\* Hutchison (*Journ. of Physiol.*, xx, 1896; *Brit. Med. Journ.*, 1897, 194) states that the active part of the thyroid is found in the colloid substance which he finds is precipitated by acetic acid and redissolved by excess of acid. From chemical experiments performed since the publication of his papers, I find that after precipitating cold alkaline aqueous extracts of fresh sheep's thyroid with acetic acid, some of the substance that palliates the cachexia still remains in solution. When this solution is further treated with acetate of lead, etc., this substance finally appears as one of the probably many components of the waxy mass of extractives. The greater the quantity of this substance in the waxy mass, the more active the mass of extractives seems to be. The presence of this substance in the mass that I have termed "extractives" possibly accounts for the different results that I have obtained by injections of the so-called extractives.

In rabbits and monkeys I have not been able to produce toxic symptoms by administration by mouth of colloid from perfectly *fresh* sheep's thyroid, although large doses were given. The colloid was obtained in the manner Hutchison describes, but the *fresh* glands were extracted only for one hour. The toxicity of colloid after an 18 hour extraction of the *fresh* glands has not been tested, but from Hutchison's account of the rise of temperature, etc., after doses as small as .05 gm., its toxicity can evidently be considerable. (January, 1898.)

Only the perfectly fresh glands were used after being carefully examined for accessory thyroid bodies and were treated preferably by the acetate of lead method, which was also employed in the extraction of creatin and other constituents of muscle, and which Fraenkel \* first recommended for the extraction of the substance that he calls "thyreο-antitoxin." All of the methods that have been given for the extraction of the fresh thyroid apparently yield the active substances to which I refer in this section, but the acetate of lead process has always proved the least laborious and yielded considerable quantities of the waxy mass, consequently I have usually employed it.

Whether or no the process recommended by Drechsel for the extraction of his two thyroid principles will yield from the thymus substances that possess a physiological activity which is similar to that possessed by the products which I have obtained from the thymus by the acetate of lead method, I am not at present able to state positively. So far I have applied Drechsel's method to the thymus but twice. As in the case of the thyroid, the method yields with the thymus gland two brownish oily products, both of which contain numerous varieties of crystals. Many of these crystals are evidently identical with those that the thyroid gland yields by Drechsel's method, if one judges by their microscopical appearances only, but it must be admitted that I have met with no better success here in obtaining either these thymus crystals or the mother liquor in an absolutely pure state, than I did when the fresh raw thyroid gland was extracted according to Drechsel's method. One of these mixed crystalline products evidently possessed considerable activity, but the results with the other are not at all conclusive, for the five thyroidec-tomized dogs to which the substance was given proved at the autopsy to have moderately well developed accessory thyroid glands in the aortic region. Further experiments at present in progress will be necessary before I can conclude definitely whether both of the products that the method yields possess activity.

Precipitation of a solution of the brown gummy mass of thymus extractives that results from the acetate of lead method, by phospho-molybdic or by phospho-tungstic acid, boiling the precipitates first in water and then in an ammoniacal solution, decomposing with baryta, etc., appears to yield with the thymus also two active portions containing sundry varieties of crystals which deposit from the mother liquor. Whether the active principles of the two portions are really two chemically different bodies or not I am not prepared to say in this preliminary

\* Fraenkel, loc. cit.

notice of the phenomena that I have observed to follow the injection of these thymus extractives. If the active principles be two different chemical bodies, both are evidently contained in the gummy waxy mass which the thymus, like the thyroid, yields by the acetate of lead process. This waxy mass of mixed thymus extractives has, therefore, usually been employed, and the following remarks refer to its effects unless it is specifically stated to the contrary.

Before entering into the description of the actual experiments with the thymus extractives I deem it pertinent in this connection to state briefly that at the outset of the experiments a filtered thymus bouillon was employed. This of course was concentrated by boiling, filtered, and a large quantity injected into two thyroidless dogs that presented marked prodromal symptoms of an impending dyspnœic attack, considerable conjunctivitis and rhinitis, enophthalmos, frequent muscular twitchings, and considerable stiffness in the hind legs. In about one hour both animals appeared to be improved, for the symptoms of the impending attack subsided. Owing to the lateness of the hour no further observations of the animals were made until the following morning, when it was found that the thyroidless dog that had received the concentrated bouillon to the amount of 122 ccm. (= 244 grm. of fresh thymus) was lying sprawled out and apparently paralyzed in the hind legs, but the dog was still able to elevate itself upon the forelegs and drag the rear part of the body along for a short distance in a very unsteady manner. When the animal attempted to move, slight muscular twitching appeared in the forelegs, but when the dog was lying quietly no tremor could be detected. The conjunctivitis and rhinitis had almost subsided and the eyes no longer appeared sunken. The animal took its food with fair appetite and recovered the use of its rear legs by the afternoon. The following morning the dyspnea returned and the dog had several convulsions, dying about 98 hours after the thyroid glands had been excised. A most careful post-mortem examination revealed no accessory bodies.

The second dog that received but 55 ccm. of the thymus bouillon was also apparently much benefited by the injection, for the signs of impending dyspnea gradually subsided and on the following morning were entirely gone. On this and on the two succeeding days the existence of the cachexia was only manifested by occasional fibrillary twitchings in various muscles, progressive emaciation, periods of restlessness, moderate conjunctivitis and the manifestation of but little desire for the food that was placed before the animal. On the fifth day the typical fits occurred in the very emaciated animal, and death followed about midday. At the autopsy several small accessory bodies were found in the aortic and in

the thyroid regions, consequently this experiment indicated in my opinion absolutely nothing.

Although no accessory bodies were found in the former dog, a definite conclusion regarding the cause of the apparent, though temporary, diminution of symptoms could not of course be made from such imperfect data. Nevertheless, the peculiar experience was considered worth a more extensive and careful investigation, and the following description of one of my experiments, it is hoped, will convey some idea of the effects which I have repeatedly observed to occur in totally thyroidless young dogs after the injection of a boiled 4 or 6 per cent. aqueous solution of the waxy mass of thymus extractives.

Two young, healthy dogs, respectively weighing 8200 grm. and 5750 grm., were chosen. The thyroid bodies of both were quickly removed (ether anaesthesia) with strict aseptic precautions. On the following morning the injections were begun, dog (*a*) receiving a small dose of the thymus extractives; dog (*b*) a similar amount of a 4 per cent. solution of thyroid extractive which had been extracted from fresh raw thyroid, also by the acetate of lead method.

The condensed notes of the experiment are as follows:

Dog (*a*). 1st day: 19 minims of a 4 per cent. thymus solution injected.

2nd day: 38 minims were given, as the animal was very restless and the usual tetany and conjunctivitis were beginning to appear.

3rd day: 87 minims given in three doses during the day, as the limbs of the dog were very stiff, and a mild attack of dyspnoea occurred. Weight 8005 grm.; pulse 160; temperature  $105\frac{2}{3}^{\circ}$  F. The animal still takes its food.

4th day: 90 minims injected; dog exhibits considerable tremor.

5th day: 9.45 A. M. 30 minims injected. Animal much brighter and is able to run about, although fibrillary twitchings still appear in the hind legs. Weight 8300 grm.; pulse 140; temperature  $103^{\circ}$ . Conjunctivitis subsided. The animal begins to take but little food. 1.30 P. M. Symptoms of an impending dyspnoëic attack being present, 50 minims were injected and repeated at 2.30 P. M., after which the distressing symptoms quickly subsided.

6th to 9th day: 125 minims per day injected in three doses. Dog very weak and emaciated. Weight 7400 grm. Has refused all food for the past two days and has to be given milk by means of the stomach tube. Most of the milk was vomited ten or fifteen minutes after the withdrawal of the tube. Had a slight fit on the ninth morning, but recovered shortly after the injection of 50 minims of the thymus solution.

10th to 13th day: 75 minims per day injected. Weakness and emaciation steadily increasing and no food is retained in the stomach.

14th day: 50 minims given, but the dog died about noon. Weight of animal at death 5800 grammes.

Autopsy: The most careful search failed to reveal the presence of accessory thyroid tissue.

Dog (b). Was given 19 minims per day of a 4 per cent. solution of the thyroid extractives until the morning of the fourth day, on which date the dog was found at 9.15 A. M. having a series of very severe fits. After several doses, in all equaling 104 minims, the dog recovered and in the course of two hours was able to run about, although a moderate degree of stiffness still persisted in the hind legs. From the 4th to the 9th day, on which the animal died, 25 to 100 minims of the thyroid solution were injected per day, the amounts depending upon the condition of the animal. During this period several slight dyspneic attacks occurred, but no more general convulsions. From the 4th day the dog refused food, and from this day the progressive emaciation became more prominent, 950 grammes being lost in all. At the autopsy a small accessory thyroid body about 2 mm. in diameter, which contained a small quantity of colloid, was found in the aortic region.

In this instance the animal injected with the thyroid extractives for comparison died before the animal injected with the thymus. Generally both animals survive for fifteen or eighteen days, but not longer unless they possess a small amount of accessory tissue.

Normal dogs injected with equal amounts of the above solutions apparently exhibit no effects.

The results of the foregoing and of other similar experiments lead me to believe, after comparing the effects of the thymus extractives prepared by the above method, that the concentrated thymus extractives prove just as efficient in palliating the cachexia in totally thyroidless dogs as the thyroid extractives.

I do not desire that the above statement should be interpreted as meaning that the active extractives from the thymus and the thyroid are chemically the same. This question has not at present been answered. It is possible that extracts from other cellular organs may be found to modify the acute cachexia as well as the extractives from the thyroid gland.

I am fully aware that the periods of survival of the thyroidless dogs that were injected with the thymus extractives or even with the thyroid extractives, to which the following remarks likewise apply, will seem entirely too short if my results are compared with those of other observers who claim to have kept thyroidless dogs alive for many weeks or even months by the mere administration of thyroid material by the mouth. I have, however, previously called attention to the fact that although I have repeatedly given enormous amounts of raw thyroid or of the extractives from the fresh thyroid to large numbers of thyroidless dogs, I have never been able to obtain such results except in dogs that were invariably proved at the autopsy to have several accessory thyroid bodies, microscopic or larger in size. Whenever the supposedly thyroidless young dogs continued to take food with their usual appetites, or when the profound disturbances of nutrition failed to appear fairly rapidly, or, if at the end of twenty days the thyroidectomized dogs that were being subjected to the thymus or to the thyroid treatment appeared to differ but little from normal dogs, I always concluded that a certain amount of living and functionally active accessory thyroid tissue was still present in the dogs. At the autopsy the animals were examined most carefully in all those regions in which Piana, Fuhr, Wölfler, Wagner, Carle and others have stated accessory thyroid bodies could occur. As a result of this examination it was found that my opinion was invariably correct, and consequently it was not allowable to conclude that the extractives or the diet of raw gland were responsible for the modified cachexia and long survival of the dogs in a practically normal condition. Even the activity of the small accessory bodies is not always sufficient to keep the dogs alive, and in spite of the aid derived from large amounts of thyroid material, some dogs will die as early as the tenth or fifteenth day. In other dogs the activity of the inaccessible aortic accessory bodies seems to be greater, and aided by the thymus extractives, the thyroid juice, etc., the lives of the dogs may be so prolonged that the remaining accessory bodies become moderately hypertrophied and sufficiently functionally active to maintain, with the assistance of the injected thyroid juice or extractives, the metabolism of the dogs in a fair state of equilibrium for a number of weeks or even months. Usually, however, the thyroid function seems to be but imperfectly assumed by the remaining accessory bodies, and in spite of the injections the profound disturbances of nutrition appear and ultimately bring about a fatal termination. If all thyroid tissue has been removed from the dog it is impossible, as I have remarked elsewhere, to prevent the comparatively early occurrence of this fatal termination, even though enormous doses of a thyroid prep-

eration that has proved to be extremely efficient in myxedema be given. Further, the unintentional oversight of the smaller and evidently active accessory bodies (Plates XV and XVI) which frequently are recognized only with extreme difficulty, especially when they are located in the mediastinum, fully explains, according to my experience at least, the discordant opinions existing at present among the various previous observers who have administered thyroid to young thyroidectomized dogs.

As is indicated in the preceding remarks on the possible behavior of thyroidectomized dogs owing to the presence of small, and often unsuspected accessory bodies, it is very essential to definitely determine their presence or their absence before a true comparison of the influence which the derivatives of the thymus gland appear to exert on the cachexia can be made with that resulting from the employment of the thyroid gland or its concentrated extractives.

Naturally the knowledge of the above-described effects on the cachexia of the concentrated thymus extractives is not of service in explaining the functions of the thymus gland, but it may furnish a partial explanation for the favorable results that Owen, Mikuliez, McKie, myself and others have observed to occur in patients with exophthalmic goitre when the thymus method of treatment was employed. I write "a partial explanation," for, besides the effects possibly directly due to the action of the extractives, the beneficial effects resulting from the daily ingestion of a large amount of very digestible and easily assimilable food, such as the sweetbread is, must also be taken into consideration. Granting, however, that the beneficial results that have been noticed in exophthalmic goitre are due to the extractives, the following question at once presents itself: "How can substances such as the thymus extractives which, as I clearly show, strikingly palliate the symptoms of the acute cachexia in dogs, possibly benefit the symptoms of a disease that are stated by the majority of observers to become generally more pronounced when the usual thyroid preparations, presumably also containing principles which palliate the acute cachexia, are ingested?" Or, in other words, if the thyroid extractives and the thymus extractives are equally capable of temporarily supporting an animal that has been deprived of all thyroid function, how can the administration of these similarly acting extractives give rise to beneficial effects in a diseased condition which the present consensus of opinion states to be the result of increased thyroid activity?

To attempt to properly elucidate these questions would entail necessarily a detailed discussion of the entire and still unsettled thyroid theory of Graves' disease. The pros and cons of this theory have been

discussed so fully within recent years by numerous writers that it is not necessary to set them forth in detail in this paper.

In spite of all the evidence brought forward to support the theory that the symptoms of Graves' disease arise in consequence of exaggerated thyroid function, it is nevertheless an admitted fact that cases exist that present besides symptoms of exophthalmic goitre many of the characteristics of myxoedema. But it is reasonably conceivable from the facts which the observations of Reverdin, Schiff, Kocher, Fuhr, Horsley, von Eiselsberg, Poncelet, Zesas, Ord and others have brought to light concerning the sequence of the various groups of symptoms which arise as the result of the total suppression of the thyroid function, or even from a partial interference of its function, that this class of patients ought to exhibit, before the myxoedematous tissue changes occur in the myxoedematous variety of Graves' disease, some of the symptoms at least that arise during the earlier stages of the cachexia that supervenes when the thyroid function is sufficiently diminished. The most prominent early symptoms, or symptoms of the *neurotic* stage (Horsley) as taught by experiments on monkeys, dogs and other lower animals, as well as by the sequence of events that has been observed after operative myxoedema (Reverdin) in men, consist of the following: profound general weakness, restlessness and periods of depression, fibrillar muscular tremor (rate of clonus 8-10 per second), local and general tetanoid spasms, emaciation and anaemia, increased lachrymal, nasal and salivary secretion, accelerated heart-beat, moderate or considerable elevation of temperature (Herzen, Horsley, Ughetti, Alonzo, Rogowitsch, Formánek and Haškovec), dyspnceic crises, ocular disturbances, such as enophthalmos \* with narrowing of the palpebral fissure, muscular palsies, corneal and conjunctival congestion, etc., lowered blood pressure, and frequently dilated peripheral arterioles manifested by the congested condition of the various mucous membranes. Later, trophic disturbances of the hair, nails, etc., occur, and various motor palsies are also not infrequent.

When the myxoedematous tissue changes have fully developed it has

\* In two of my meat-fed thyroidless dogs with accessory aortic thyroid bodies, in one bread-fed partially thyroidless dog, and in a dog in which all the vessels of both thyroid lobes had been ligated with catgut, a considerable prominence of the eyes developed on the morning after the thyroideectomy. The exophthalmic condition was so noticeable that, before I had an opportunity to direct attention to it, it was previously remarked by a laboratory attendant and others who had not been forewarned to be on the lookout for the occurrence. In the latter dog the exophthalmos lasted for about two weeks, but gradually became less noticeable owing to the marked puffiness that developed in the dog's face.

been clearly demonstrated both in man and in the lower animals that a reversal of the majority of the above symptoms is the rule; hebetude, increased weight, diminished secretions, subnormal temperature, slowed heart-beat, etc., thus replacing their opposite of the early stages of the cachexia. The exophthalmos with narrowing of the palpebral fissure, however, generally persists.

Thus a great many of the symptoms proper to the early stages of the cachexia are, as Mackenzie, Gley and others have specially pointed out, strikingly like those that are usually manifested by the average patient with exophthalmic goitre. Therefore, if one considers the following facts, namely, (a) the remarkable similarity of many of the common symptoms of exophthalmic goitre to those of the first stage of the cachexia thyroidectomica; (b) the well-known tendency of the struma to become indurated or otherwise degenerated, and the clinical fact that the partial removal of the supposedly overacting struma from individuals that manifested only the usual symptoms of exophthalmic goitre, unaccompanied by myxœdematous tissue changes, has been followed by the appearance of pronounced tetany, general or local swellings, myxœdematous in character, and occasionally by death preceded by all the usual symptoms of the acute cachexia, one is led to the conclusion that an insufficiency of at least one of the various possible functions of the thyroid gland may be present in exophthalmic goitre without myxœdematous tissue changes being evident.

Thus, from our knowledge of the effects of the fresh thymus and thyroid extractives on the symptoms that result from an insufficiency of thyroid activity, it is but reasonable to expect that, if a partial "hypothyroidation" (Gley) is directly or indirectly responsible in many cases of Graves' disease, an exhibition of these extractives by mouth would probably be followed by more or less amelioration of many of those symptoms. My previously described observations with the fresh thymus and thyroid glands confirm this, but my observations with the dried thyroid preparations conform with the usual experiences of others, that the exhibition of the average commercial thyroid preparations, powders, concentrated extracts, etc., to patients who have Graves' disease but no evidence of myxœdematous changes, generally results in making the patients decidedly worse, an occurrence that is due, in my opinion, to the action of the toxic alteration products which further disturb the already disordered metabolism.

Typical myxœdematous patients are also well known to be very susceptible to the poisonous preparations, many well-known English, German, French and American observers advising extreme caution in the

thyroid treatment of myxoedema, and in fact, as Ewald has pointed out, symptoms of intoxication may develop during the treatment of myxoedema without the appearance of any curative or beneficial effects. Sonnenburg's\* experience which was related in the historical retrospect (Part I) clearly shows, however, that in operative myxoedema enormous quantities of thyroid may be taken without development of any toxic symptoms whatever, the patient even improving and gaining in weight from the very commencement of the treatment.

These observations, coupled with various facts described in connection with the symptoms that occur in rabbits and monkeys after the administration by mouth of the concentrated toxins extracted from stale or dried thyroid, clearly indicate that the prominent katabolic effects which rapidly occur in myxedema, obesity, etc., in consequence of the ingestion of the average dried thyroid preparations, are probably almost entirely due to the action of the contaminating and varying cadaveric poisonous derivatives.

Further, one is almost led to believe that, although an increased susceptibility to these toxins exists in myxedema, if very small amounts of these powerful stimulators of katabolism are given in combination with those thyroid substances which are capable of modifying the early symptoms of the acute cachexia, the myxoedematous individual may be somewhat benefited by the reduction in weight from the decided destructive metamorphosis of the myxoedematous tissues and the increased excretion, which the toxic varieties of the thyroid preparations have been experimentally shown to produce. I am not, however, in a position to substantiate the above by clinical proof, for, as has been already stated, the palliative and the toxic activities of the thyroid preparations in general use, at least in the United States, are so variable and the clinical reports describing the various effects that have followed their ingestion in large and in small doses are often so conflicting regarding the occurrence of early and pronounced destructive metamorphosis and consequent loss in weight in myxoedematous individuals who are being treated with the different thyroid preparations, that it is impossible to gain a more extensive information from those clinical reports than the following:

- (1) The majority of myxoedema cases are improved by the various preparations, the improvement being accompanied by a considerable loss in weight and other trophic manifestations.
- (2) Considerable improvement occurs, but even a gain in weight accompanies the improvement.

\* This observer evidently employed thyroid material in which no appreciable post-mortem alteration had occurred.

(3) Loss in weight accompanied by the appearance of very distressing symptoms follows, but no evident improvement in the various nervous and other symptoms of the myxœdema.

(4) Neither loss in weight nor improvement in any of the symptoms results until a number of different thyroid preparations have been tried.

These facts are extremely contradictory, but the above-mentioned possible dual activity of the average thyroid preparations will probably be found to be mainly responsible for the irregularities described by clinicians in connection with the treatment of myxœdema by the thyroid method.

As the results under the fourth heading indicate, some of the preparations contain scarcely any of the active extractives, although an examination of a large number of samples of various thyroid preparations, some of which proved absolutely inactive in palliating the acute cachexia in totally thyroidless dogs, showed that iodine, and presumably the thyro-iodine of Baumann and Roos, was present in considerable amounts in all of those preparations of the crude powdered gland. This substance may, as Roos states, be the substance which causes the reduction in the goitre, but reduction in the goitre will also follow the ingestion of the thymus gland (Owen, Mikulicz and myself), and the thymus contains, according to Baumann,\* no iodine, a fact that has been repeatedly verified by myself. Other organic bodies besides thyro-iodine consequently possess the property of causing the struma to diminish. In fact, the recent experiments of Gottlieb † with the concentrated thyro-iodine prepared according to Baumann's method are confirmed by my own experiences with the inactive or, rather, non-palliative iodine-containing thyroid preparations. The conclusion reached by this writer, that the thyro-iodine is not capable of keeping thyroidless dogs alive, is in my opinion perfectly correct, and from his experiments I judge that chemically pure thyro-iodine does not influence in the least ‡ the cachexia in *totally* thyroidless dogs.

\* Baumann, loc. cit.

Baumann (*Münch. med. Woch.*, 1896, No. 14) states, since the above was written, that by using large amounts of calf thymus he was able to detect the presence of iodine in it, although the quantity of iodine was very small. I have repeatedly tested the thymus for iodine, but it did not appear to be present in any of the thymus glands that were tested. I have, however, found traces of iodine twice in the salivary glands of the ox, and once in the parotid gland of the dog. Possibly accessory thyroid bodies were present in the thymus tissue examined by Baumann.

† *Deutsche med. Wochenschr.*, Apr. 9, 1896.

‡ Experiments performed by me since this paper was sent to the editor show that some lots of commercial iodo-thyrine (Bayer) do moderately

Is the presence of all the principles which Fraenkel, Drechsel and Baumann describe necessary in a thyroid preparation in order that the preparation may produce all the good effects that one observes in myxoëdema? This remains to be seen, for further very carefully conducted observations are certainly necessary to determine this point. And in conclusion I add that only absolutely pure principles should be employed for such observations.

#### *E. The intravascular injection of the extractives.*

##### *1. Intravascular injection of boiled thyroid extracts.*

While some of my subcutaneous injection experiments were in progress, Oliver and Schäfer \* having investigated the blood pressure effects of intravascular injections of decoctions of various glands, published the fact that the intravascular injection of the thyroid extract gives rise to an almost immediate but transient diminution of the blood pressure by increasing the calibre of the arteries, although the beats of the heart remain at about the same rate and of the same strength. Schäfer † states, "Before this effect upon the blood pressure was discovered by us it had been shown by my co-worker, Dr. George Oliver, that the exhibition of thyroid juice and other preparations of thyroid in the human subject has a tendency to increase the calibre of the radial artery." Haškovec ‡ also noted the fall of the blood pressure and, besides, an acceleration of the pulse. Fraenkel tested the effects of intravascular injection of his thyro-antitoxin and found that this produced an acceleration of the pulse but no fall of blood pressure. Nor does a cold aqueous extract of dried sheep's thyroid produce, according to this observer, a fall of blood pressure, although an increase in the number of heart-beats does occur. Fraenkel states that he does not understand this difference and, therefore, gives no explanation.

influence the cachexia in totally thyroidless dogs and monkeys when the dose is rather large; other lots do not appear to affect the course of the cachexia at all, although the preparations contain iodine. When solutions of these active iodo-thyrine preparations were treated according to Drechsel's method they yielded small amounts of two brownish gummy substances, neither of which contained iodine. Injections of a mixture of these gummy substances into totally thyroidless dogs palliated the cachexia somewhat, but not as much as boiled aqueous extracts of fresh raw thyroid usually do.

\* *Journal of Physiol.*, xvi, 1894; xvii, 1895, and xviii, 1895.

† *Internal Secretion, Brit. Med. Journal*, 1895, 341.

‡ Haškovec, quoted by Fraenkel, loc. cit.

In order to compare the blood pressure effects produced, according to Schäfer and Oliver, by aqueous decoctions of fresh raw or of dried thyroid material with those produced by the thyroid extractives which I had found capable of modifying the acute cachexia thyroideotomica in dogs, I repeated Schäfer and Oliver's experiments, and also performed a number in which the various thyroid and thymus extractives were employed. My results with the decoctions prepared from absolutely fresh sheep's thyroid and from calf thymus practically agree with those of Schäfer and Oliver; the injections produced, as these observers have pointed out, a transient fall in the blood pressure, although the strength of the heart-beats and the rate remained almost the same (Plate XVII, Fig. 1). I state "almost," for usually the rate diminished by three or four beats per minute, and frequently, if a very large amount of the concentrated neutralized decoction was injected, a diminution of eight or ten beats occurred. This slight diminution in rate, like the fall of the blood pressure, was but temporary, and as the blood pressure gradually returned to its original height, the number of the heart-beats became the same as before the injection. Although the above effects were striking when fresh decoctions of the fresh raw material were injected into the external jugular of etherized dogs,—especially when both vagi had been cut,—I found that if the decoctions were kept and allowed to become more or less putrefied, their effect upon the blood pressure became markedly less and less and finally disappeared.

In several different anaesthetized and morphinized dogs a number of reboiled and filtered thyroid and thymus decoctions in various stages of decomposition were injected, and though the majority of them produced scarcely any diminution in the blood pressure, several of the stalest solutions caused a transient acceleration of the heart-beat. In no instance, however, did the acceleration amount to more than eight beats per minute when 2 ccm. (= 2 grm. of raw gland) of the stale solution were injected.

When the dried commercial thyroid preparations were employed as the source of the decoctions—of course allowing the pulverized gland to macerate for a number of hours before boiling the mass—my

results were not nearly so striking or so uniform as those produced by smaller doses of the decoction of fresh raw thyroid which had not been allowed to macerate more than a few minutes before boiling.

To sum up the cardiac and blood pressure effects of these decoctions of stale and dried material:

(1) Some produced a moderate fall of blood pressure, the number of heat-beats remaining unaltered or occasionally diminishing three or four beats per minute. The fall of the blood pressure was always more prominent after both vagi had been divided.

(2) Some produced apparently no effect at all.

(3) Some caused no fall of blood pressure, but the frequency of the heart-beat temporarily increased six to ten beats per minute when the vagi were intact.

(4) A decoction from one preparation invariably gave a faint increase in the blood pressure in a morphinized dog with both vagi cut.

Thus, in (1) it will be seen that the effects practically agree with those that, according to Schäfer and Oliver, the decoctions from the dried preparations produce, while in (3) the effects correspond with those that Fraenkel states are produced by the "thyreo-antitoxin." The results in (2) depended upon the probable absence of or, as I found subsequently after extracting large quantities of the dried preparations, upon a considerable decrease in the relative quantity of substances that produced the fall of blood pressure. In (4) it was clear that considerable alteration of the constituents of the preparation had occurred, a fact that was further proved by the failure of injections of the decoction to modify in the least the symptoms of the acute cachexia in totally thyroidless dogs.

## *2. Intravascular injection of the thyroid and other extractives.*

Extending the above blood pressure tests to the ultimate thyroid and thymus extractives, the following extractives isolated by methods previously described in detail (page 203 et seq.), were tested and found invariably to produce a very decided fall of the blood pressure when they were injected directly into the external jugular vein or into the portal circulation. Briefly, these substances were:

(a) Solutions of the brownish, waxy, impure mixed extractives, obtained from perfectly fresh thyroid and thymus glands, by the three methods which we have described.

(b) The brownish waxy mass obtained from the waxy mass by treatment with 90 per cent. alcohol and repeatedly precipitating the alcoholic solution by anhydrous ether. (That from the thyroid should correspond to Fraenkel's thyreo-antitoxin.)

(c) The two crystalline mixtures obtained by decomposing the waxy thyroid mass with phospho-molybdic acid, etc.

(d) The two crystalline mixtures obtained by decomposing the waxy thyroid mass with phospho-tungstic acid.

(e and f) The crystalline mixtures from the thymus as in c and d.

(g and h) The two crystalline mixtures from the fresh raw thyroid by Drechsel's process.

(i and j) The two crystalline mixtures from the fresh raw thymus by Drechsel's thyroid process.

Intravascular injections of each of the above extractives produced effects practically similar to those produced by the decoctions of the fresh raw material (Plate XVII, Figs. 1 and 2, and Plate XVIII, Fig. 3). When neutral 5 or 10 per cent. solutions were employed the fall of the blood pressure was very pronounced, and frequently the number of heart-beats very perceptibly, though temporarily, diminished.

When the dried commercial thyroid preparations were used as the source of the extractives, it was found that generally the extractives from them affected the blood pressure in just the same way as the extractives from the fresh thyroid, but relatively small amounts of the extractives were yielded by most of the dried preparations, a circumstance which probably accounts for the results in (2) with the more dilute decoctions of those dried preparations.

Two especially foul-smelling dried preparations were extracted by the acetate of lead method, and the resultant waxy mass of extractives treated by alcohol, ether, etc., the cleaned ether precipitate dissolved in normal saline solution and injected. No fall of blood pressure followed, but a slight acceleration of the heart-beat (about eight beats per minute) was produced several times when 1.5 ccm. of a 5 per cent.

solution were injected at one dose. This acceleration, however, was often variable and frequently did not occur when the above quantity or even a larger quantity (2.5 ccm.) was given.

Comparing these effects with those produced by the various extractives obtained from the absolutely fresh raw thyroid, it was evident that the blood-pressure-reducing substances had undergone considerable alteration in many of the dried thyroid preparations. Why I cannot say, for I do not know exactly the process by which the dried preparations were prepared, nor do I know how long a time was consumed in the slaughter-houses in gathering the raw thyroid glands together for the various manufacturers of the desiccated preparations.

I have stated that if the extractives (*a-h*) were injected into one of the tributary veins of the portal circulation, a diminution of the blood pressure resulted, but, as might be expected, the abrupt fall that usually occurred when the injection was made directly into the external jugular vein was not produced. The total fall, however, was about the same as if an equal dose had been injected into the jugular vein.

Wishing to see if the extractives would affect the blood pressure after being absorbed through the stomach and the intestines, I injected into the stomachs of etherized fasting dogs very large quantities of the extractives *a, c, d, e* and *f* mixed, a mixture of *g* and *h*, and also one of *i* and *j*, but without producing the least diminution in the blood pressure.

The method pursued was as follows: After previously inserting a cannula into the carotid artery and determining the blood pressure, 15 grammes of *a* were injected into the empty stomach of a dog weighing 4300 grms., and a continuous tracing was taken during a period of two hours and twenty-five minutes, and except for slight irregular changes that one usually sees occur with prolonged anaesthesia, no alteration of the blood pressure could be detected. Had the same amount been injected directly into the external jugular vein almost instantaneous death would probably have occurred, for I have seen it occur when even 5 grms. of *a* from the thyroid or from the thymus were injected in this manner. Such enormous quantities appear to cause very profound chemical alterations of the blood.

Extractives from the aqueous decoctions of certain other glandular organs, for example the submaxillary glands of the ox, also produce a fall of the blood pressure, and so do the extractives yielded by muscle, and also those from commercial meat extracts. The effects of injecting one derived from an ordinary sample of Liebig's extract by the acetate of lead method are shown in Plate XVIII, Fig. 4, and no further comment is necessary except the remark that a transient increase of eight to ten heart-beats was usually produced by these injections.

From the above experiments it is evident that Fraenkel's thyreo-antitoxin, which, he states, does not lower the blood pressure, and the active palliative substance which I obtain from the perfectly fresh raw thyroid gland by the process that he recommends, possess entirely different properties in respect to their influence on the blood pressure and on the rate of the heart. The cause of this difference is at present inexplicable, unless it be that the dried thyroid material which he employed as the source from which he extracted his thyreo-antitoxin had undergone extensive alteration during its manufacture. The blood pressure effects that I have got with the thyroid extractives by Fraenkel's method, that is to say, with the extractives of those preparations that had undoubtedly modified the acute cachexia in totally thyroidless dogs, and with the two substances obtained according to Drechsel's methods, practically accord with those that Schäfer and Oliver noticed with the aqueous decoction of the raw glands.

But, though the transient fall of the blood pressure and tendency to slow heart-beats are produced by the intravascular injections of thyroid decoctions and extractives, this fact does not conclusively prove that the hypothetical internal secretion of the thyroid gland in the living body is necessarily continually affecting the blood pressure in this manner. On the contrary, injections of large amounts (30-50 ccm.) of the defibrinated blood from the inferior thyroid vein removed while the gland was being massaged, did not produce during any of my experiments the above effects; a fact which would of course only tend to show that none or, if any, an extremely small quantity of the blood-pressure-reducing thyroid substance was present in the blood after passing through the gland.

Nor can one assert from the results of the preceding experiments with the thyroid derivatives that the transient blood-pressure effects

that are produced are specific for the secretion of the living thyroid gland, for derivatives that produce similar effects on the blood pressure may be readily obtained from the salivary glands (ox), from the muscles, etc. The latter certainly have no internal secretion, in the proper sense of the term, and with respect to the muscle extractives that lower the blood pressure the only definite fact my experiments indicate is that the proteid-free muscle extracts contain certain chemical substances that possess the properties of temporarily reducing the blood pressure when they are directly introduced in minute quantities into the circulating blood. They certainly do not indicate that these substances normally enter the blood or the lymphatics from the muscles in this particular chemical state.

Nor are the blood pressure and cardiac effects which the complex juice of the minced raw thyroid temporarily produces, when it is injected directly into the circulation, definitely apparent in exophthalmic goitre, in which disease too much internal thyroid secretion is claimed by many as being the chief pathogenic factor. Thus, in exophthalmic goitre, acceleration of the heart-beat is, according to all observers, usually the first of the cardinal symptoms to appear. Regarding the state of the blood pressure clinicians differ greatly, and from the fact that so many diametrically opposite opinions have been expressed, it is evident that the state of the blood pressure is not always the same in different patients with exophthalmic goitre. For instance, François-Franck and Marie have found the state of the blood pressure in Graves' disease to be normal.\* Sachs † states that "it is owing to the increased tension that hemorrhages are frequent from the nose." Starr ‡ writes, "The condition of the heart and arteries in myxœdema is exactly the opposite of the condition found in exophthalmic goitre. In all the cases of myxœdema a slow pulse is present. The pulse is also small and of high tension in myxœdema. In sharp contrast to

\* François-Franck and Marie, quoted by Chamberlain. *Maladie de Basedow*, Paris, 1894.

† B. Sachs, *Nervous Diseases of Children*. New York, 1895, p. 199.

‡ M. A. Starr, *Med. News*, April 18, 1896.

this condition are the rapid, full, large pulse and throbbing arteries constantly complained of in exophthalmic goitre.” \*

On the other hand the removal of the thyroid gland in dogs and monkeys is followed by a state of subnormal blood pressure, the very condition that the injections of thyroid decoction temporarily produce. In both varieties of animals the pulse-rate is accelerated also in the early stages of the cachexia, but as the myxœdematous stage supervenes (monkeys, rarely dogs), the number of heart-beats becomes reduced and falls usually below the normal.

It is evident, therefore, that many complicating and, at present, inexplicable factors are probably concerned in the vascular manifestations that arise in consequence of disturbed thyroid function. All that one can say from experiments of the foregoing character is, that the connection between the effects observed after the intravascular injections of aqueous extracts, etc., of ground thyroid tissue, and the various cardiae and vascular manifestations observable in conditions in which the supposed internal thyroid secretion is presumably disturbed, is entirely too indefinite in the present state of our knowledge of the presumed internal secretion to warrant the inference that the internal secretion of the living thyroid gland has for one of its functions the production of cardiae and vascular effects identical with those produced by the direct intravascular injection of the extracts, decoctions and various chemical derivatives of the thyroid tissues.

#### VI. CONCLUSIONS.

From the results of the various experiments already detailed I feel justified in drawing the following conclusions:

- (1) Absolutely fresh thyroid gland is not poisonous, in the usual sense of the term, when absorbed through the alimentary canal.
- (2) The symptoms of induced thyroidism are manifestations of an intoxication resulting from the ingestion of decomposed thyroid material.

\* The blood pressure of six females with well defined exophthalmic goitre has lately been investigated by me with the sphygmodynamometer of Oliver. 130 to 150 grammes were necessary to just occlude the radial arteries. The blood pressure in these patients was thus normal, 120 to 160 grammes being the normal limit according to Oliver.

ial, a conclusion that agrees in part with the previously related observations of Lanz.

(3) The so-called experimental thyroidism is not specific for the thyroid only, for the ingestion of many substances derived from animal tissues other than the thyroid gland may produce an intoxication strikingly similar in every respect to that of experimental thyroidism.

(4) Most, if not all, animal tissues yield substances which, if injected in large quantities directly into the circulation or beneath the skin, will produce an intoxication often very similar to that produced by injections of various substances derived from the fresh thyroid tissue.

(5) The effects resulting from the intravascular or subcutaneous injections of aqueous extracts, decoctions and the concentrated extractives of the thyroid tissue, of the thymus, of muscle, etc., are by no means necessarily indicative of the function and the action of the hypothetical internal secretions of the same tissues during life.

(6) The utilization of the fact that ingestion of decomposed thyroid material produces on certain occasions an intoxication with certain symptoms similar to some of those of Graves' disease is not justifiable for the furtherance of the theory that the symptoms of exophthalmic goitre result from an over-production of the thyroid secretion.

(7) Our results lead us to conclude with Drechsler that the fresh thyroid tissue yields at least probably two substances that are capable of palliating the symptoms of the acute cachexia in totally thyroidless dogs.

(8) The thymus tissue also yields one and probably two substances that are as equally capable as the thyroid extractives of palliating the acute cachexia in totally thyroidless dogs.

(9) Neither of the above substances is an enzyme, nor does either contain iodine.

(10) Neither the feeding of minced raw thyroid glands, nor the injection of aqueous thyroid extracts, decoctions, and concentrated solutions of the extracted palliative thyroid principles is capable of keeping *totally thyroidless* young dogs alive longer than a few weeks (possibly three weeks). Still less capable are the thyroid preparations containing decomposition products.

(11) The presence of one, or usually several, small accessory thyroid bodies, which gradually hypertrophy and wholly or partially assume the functions of the excised thyroid lobes, accounts for the occasionally long survival of thyroidectomized, thyroid-fed, young dogs.

(12) Totally thyroidless young dogs are so quickly overwhelmed by the cachexia, and the intervals between the thyroideectomy and the onset of the severe dyspnœic attacks and subsequent deaths differ so slightly, no matter which of the usual varieties of fresh food are employed, that *kinds* of fresh food cannot be unquestionably affirmed to influence the onset of the cachexia in any especially definite manner. Animal foods, in which constituents poisonous to rabbits have developed, probably slightly hasten the onset of the severer symptoms, and the vaunted remarkably modifying influence of a diet of ordinary milk, such as Breisacher observed, does not exist in the case of the totally thyroidless dog.

(13) Monkeys whose general metabolism is disturbed in consequence of the removal of a greater portion of the thyroid gland, evidently become more susceptible to those constituents of meat that are poisonous to rabbits, and sufficient clinical evidence exists for concluding that probably a like susceptibility to animal foods containing such constituents also exists in men when the function of the thyroid gland is sufficiently disturbed.

(14) And, finally, as regards the thyroid factor in the pathology of exophthalmic goitre, I agree with Gley that the majority of the symptoms in many patients with that disease can apparently, from an experimental standpoint, be as plausibly explained by the hypothesis of partially *deficient* thyroid activity as by the hypothesis of augmentation of thyroid function.

#### DESCRIPTION OF PLATES XIV-XVIII.

##### Plate XIV.

Photograph of a rabbit with acute thyroidism after the ingestion of 0.1 grammie of a poisonous mixture of substances extracted from a dried commercial thyroid preparation. The acetate of lead method of Fraenkel was employed to extract the dried gland, and the resultant waxy mass of extractives was washed several times with 85-90 per cent. alcohol. The

alcoholic solution was precipitated with ether, the precipitate redissolved in hot alcohol, evaporated, and after it had crystallized, dissolved in distilled water and administered through the stomach tube.

The widening of the palpebral fissure can easily be made out in the photograph. (See text, p. 211).

Plate XV.

Photo-micrographs of small aortic accessory thyroid bodies.

Fig. 1. A longitudinal section of an accessory aortic thyroid body ( $\times 24$  diams.) from a normal dog.

In the more perfectly developed portions the small vesicles contain colloid.

Fig. 2. A more highly magnified view ( $\times 300$  diams.) of a part of a section of the preceding accessory body; the structure of a portion containing no colloid is shown. The appearance is similar to that of the parathyroid body.

Plate XVI.

Fig. 3. Cross-section of an aortic body embedded in a mass of fat, from a thyroidectomized dog that had a moderate dyspnoic attack on the third day after the operation. The dog was then injected with concentrated thyroid bouillon for several days and the injections then stopped, but no return of the symptoms occurred; the dog was killed by an over-dose of ether at the end of eight days.

In the cross-section a rather unusual amount of fibrous tissue is seen between the vesicles containing colloid. Hardening in alcohol, stained with haematox. and eosin, and magnified 24 diams.

Fig. 4. Aortic body from a dog in which operative myxoedema had been produced by ligating all the thyroid vessels. As the myxoedematous swelling was beginning to subside an over-dose of ether was given and this with two other small aortic bodies was removed.

The structure of this body does not differ from that of the main thyroid lobes. ( $\times 24$  diams.).

Plate XVII.

These tracings show graphically the cardiac and blood-pressure effects that follow the intravenous injection of thyroid and other extractives; in each of the tracings the top line is that of the blood pressure, the middle line is the base line, the bottom line is the time. At — the injection was made.

Fig. 1. Showing in an etherized dog the fall of blood pressure from an injection into the ext. jugular vein of 0.5 ccm. of a neutral 20 per cent. aqueous solution of the extractives from fresh raw thyroid. The brownish wax yielded by the acetate of lead method of Fraenkel was dissolved in 85 per cent. alcohol; the alcoholic solution precipitated by ether; the precipitate redissolved several times in boiling 90 per cent. alcohol, and several times reprecipitated by ether. Finally this precipitate was dissolved in a small quantity of distilled water; the solution evaporated on a water bath at 100° C., and then set aside to crystallize over chloride of calcium. This substance, which should be the same as Fraenkel's thyreo-antitoxin, was dissolved in normal saline and injected, producing the result seen in the figure.

Fig. 2. Showing after division of both vagi the effects in an etherized dog of 2 ccm. of a neutral 10 per cent. solution of a mixture of the two partially crystalline products obtained by treating the above waxy mass of fresh thyroid extractives with phospho-tungstic acid, etc., according to the method that Drechsel recommends for the fresh raw thyroid gland. Both the crystalline products had been repeatedly cleaned by the alcohol-ether method.

Plate XVIII.

Fig. 3. Showing the effects of injecting 2.5 ccm. of a 10 per cent. solution of a partially crystallized *thymus* product. The fresh thymus glands were extracted by the acetate of lead method and the resultant waxy mass precipitated by phospho-tungstic acid. After boiling this precipitate for a number of hours, decomposing the decoction with baryta, etc., and finally cleaning with alcohol and ether, the partially crystallized product used in this instance was yielded. Morphine was given to this etherized dog.

Fig. 4. The fall of blood pressure produced by 1 ccm. of the neutralized mother-liquor from which the creatin and creatinine had been removed. Liebig's extract of meat was the source from which this mixture was obtained, and the acetate of lead method was employed to extract the creatin.







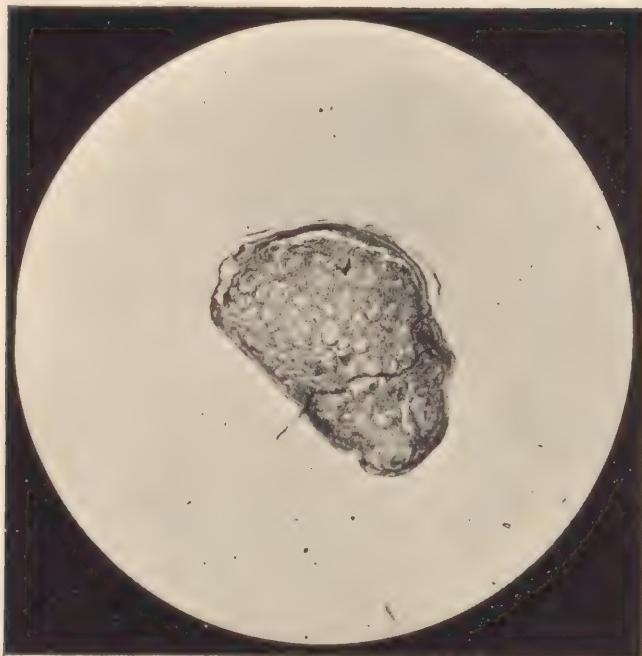


FIG. 1.

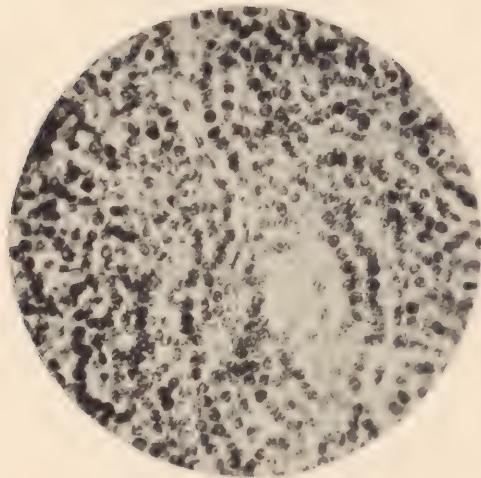


FIG. 2.



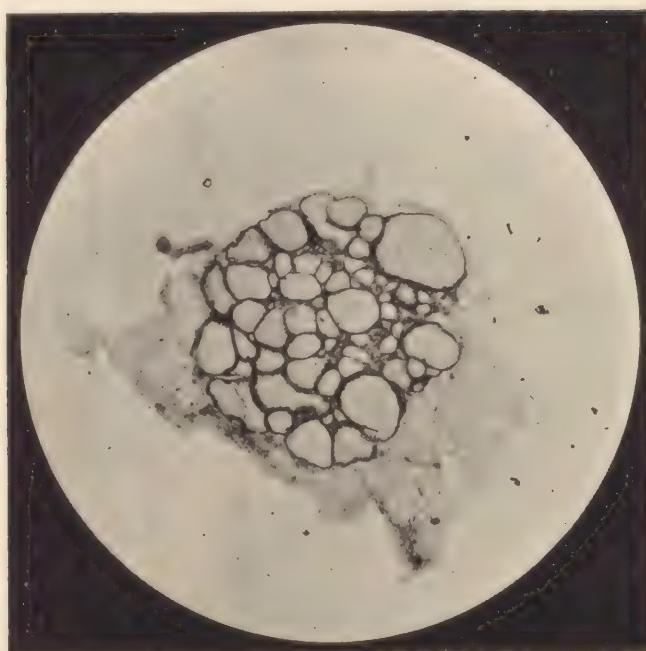


FIG. 3.

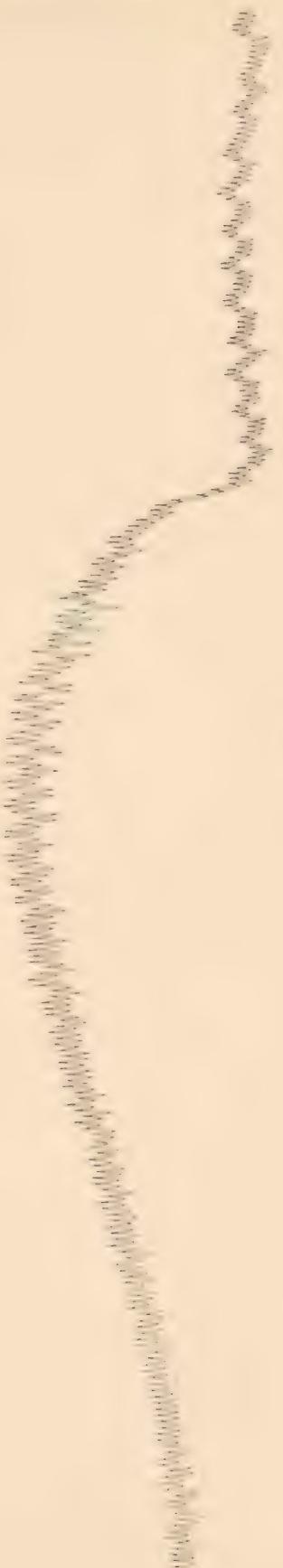


FIG. 4.



TIME: 1

FIG. 1



TIME: 1"

FIG. 2



TIME: 1"

FIG. 3



ZERO

TIME: 1"

FIG. 4

